PROTECTIVE EFFECT OF NATURAL FRUITS COMBINATION "FRUITITOP" AGAINST CYCLOPHOSPHAMIDE INDUCED TOXICITY THROUGH IMPROVING ANTIOXIDANT STATUS

Dissertation Work Submitted to

The Tamil Nadu Dr. M.G.R Medical University, Chennai

In partial fulfillment for the award of Degree of

MASTER OF PHARMACY
IN
PHARMACOLOGY

Submitted by

Mr. ASHIF.H, B. Pharm.,
Reg No: 261425662

UNDER THE GUIDANCE OF

Institutional Guide

Dr. D. Benito Johnson
Professor and Head of Pharmacology Department,
RVS College of Pharmaceutical Sciences,
Sulur, Coimbatore, Tamil Nadu.

Industrial Guide

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Associate Professor,
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Amala Nagar, Thrissur, Kerala.

DEPARTMENT OF PHARMACOLOGY
RVS COLLEGE OF PHARMACEUTICAL SCIENCES, SULUR, COIMBATORE-641-402

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CERTIFICATE

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Name and Signature of the Guide

Name and Signature of the Head of Department

Name and signature of the Dean

CERTIFICATE

This is to certify that this project work entitled "**PROTECTIVE EFFECT OF NATURAL FRUITS COMBINATION "FRUITITOP" AGAINST CYCLOPHOSPHAMIDE INDUCED TOXICITY THROUGH IMPROVING ANTIOXIDANT STATUS"** submitted in partial fulfillment of the requirements for the award of Degree of **Master of Pharmacy** in **Pharmacology** to **The Tamilnadu Dr. M.G.R Medical University, Chennai** is a bonafied work of **Mr. ASHIF.H (Reg. No: 261425662)** carried out at **Amala Cancer Research Centre, Thrissur, Kerala,** and completed the work at **Department of Pharmacology, R.V.S College of Pharmaceutical Sciences**, **Sulur, Coimbatore-641402** under my guidance and supervision and **Dr. Achuthan C Raghavamenon** (Industrial Guide) during the academic year 2014-2016 to my fullest satisfaction. This work is original and contributory.

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Date:

DECLARATION

I Ashif.H (Reg. No: 261425662) hereby declare that the dissertation entitled "**PROTECTIVE EFFECT OF NATURAL FRUITS COMBINATION "FRUITSITOP" AGAINST CYCLOPHOSPHAMIDE INDUCED TOXICITY THROUGH IMPROVING ANTIOXIDANT STATUS"** submitted to the **The Tamilnadu Dr. MGR Medical University** in partial fulfillment of the requirement for the award of **Master of Pharmacy** in **Pharmacology** is a bonafide record of dissertation work done by me under the supervision and guidance of **Dr. Achuthan C Raghavamenon**, Associate Professor, Amala Cancer Research Centre, Thrissur and under the internal supervision of **Dr. D. Benito Johnson,** Professor, Head of Department of Pharmacology, R.V.S College of Pharmaceutical Sciences, Sulur, Coimbatore during the academic year 2014-2016 and it has not formed the basis for the award of any Degree/Diploma/Associateship/Fellowship or other similar title to any candidate of any University.

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EVALUATION CERTIFICATE

This is to certify that the dissertation work entitled "**PROTECTIVE EFFECT OF NATURAL FRUITS COMBINATION "FRUITITOP" AGAINST CYCLOPHOSPHAMIDE INDUCED TOXICITY THROUGH IMPROVING ANTIOXIDANT STATUS"** submitted by **Mr. ASHIF.H (Reg. No: 261425662)** to **THE TAMILNADU DR.M.G.R.MEDICAL UNIVERSITY, CHENNAI** in partial fulfillment for the Degree of **Master of pharmacy** in **Pharmacology** is a bonafied work carried out during the academic year by the candidate at the Department of Pharmacology, R.V.S College of Pharmaceutical Sciences, Sulur, Coimbatore and was evaluated by us.

Examination Centre:

Date:

Internal Examiner **External Examiner**

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(ASHIF.H)

INDEX

LIST OF ABBREVIATIONS

%	:	Percent sign
µl	:	micro litre
^{0}C	:	Degree celsius
ALT	:	Alanine Aminotransferase
ANOVA	:	Analysis of variation
AST	:	Aspartate aminotransferase
BMC	:	Bone marrow cellularity
cAMP	:	Cyclic adenosine monophosphate
cells/mm^{3}	:	Cells per cubic millimetre
Cm	:	Centimetre
CML	:	Chronic myelocytic leukemia
CPK	:	Creatine phosphokinase
CTX	:	Cyclophosphamide
DNA	:	Deoxyribonucleic acid
DOX	:	Doxorubicin
FDA	:	Food and drug administration
Fig	:	Figure
FSH	:	Follicle-stimulating hormone
FT	:	Fruititop treated
g/dl	:	grams per decilitre
gm	:	Gram
GPx	:	Glutathione peroxidase
GSH	:	Glutathione
Hr	:	Hour
i.p	:	Intraperitonially
IU/L	:	International units per litre
KA	:	King-Armstrong
LDH	:	Lactate dehydrogenase
LH	:	Luteinizing hormone
LPO	:	Lipid peroxidation
M	:	molar

MAP	:	Mitogen-activated protein
mg/dl	:	milligrams per decilitre
mg/kg b.wt	:	milligram per kilogram body weight
mg/kg	:	1 milligram per kilogram
mg/m^2	:	1 milligram per square meter
Min	:	minute
Ml	:	Milli liter
Mm	:	Millimeter
mM	:	millimolar
mm^3	:	Cubic millimetre
MTX	:	Methotrexate
nm	:	nanometer
ND	:	Normal diet
OD	:	Optical density
RNA	:	Ribonucleic acid
ROS	:	Reactive oxygen species
RSV	;	Resveratrol
SD	:	Standard deviation
TAC	:	Triglycerides
SOD	:	Superoxide dismutase
WBC	:	White blood cells

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INTRODUCTION

Cancer is the term that denotes a group of diseases which is multifactorial in origin and characterized by uncontrolled proliferation and spread of atypical cells by distant metastases and local tissue invasion (Levy et al., 1996) causing massive aggregation. Carcinogenesis is a multi-step process (fig-1) that includes initiation promotion, conversion and finally progression phases. The growth and development of both normal cells and cancerous cells is genetically under the control and balance or imbalance of oncogene, proto-oncogene and tumour suppressor genes. The process of conversion of normal cell to neoplastic cell or called neoplastic change can be caused primarily due to physical, chemical or biological factors individually or in combination. Since, the definitive target for all these factors is mostly genetic material, cancer can be considered as a genetic disease. Nowadays lifestyle also plays important role in the development of cancer. Cancer has intrigued humanity from time immemorial and become a troubling epidemic in India. Cancer arrives silently in the life of a person with the face of innocent lump to rapidly transforming into a fatal domain. Cancer cells are genetically unstable, which causes in tumour masses development of heterogeneous cells and makes the cancer a "moving target" for drug therapy.

Current era, molecular oncology had paved its way in the comprehensive cancer care and is playing an important role especially in the discovery of novel or potent cellular targets to exploit for novel targeted treatments, identification of new biomarkers for early stage cancer detection, and to deliver a better classification of cancer for prognostication and treatment selection. The molecular basis of carcinogenesis involving genetic and epigenetic events that result in altered expression of several genes is yet not completely understood and still wants to be deciphered more, which reflects the complexity of the molecular alterations that characterize tumour cells. Cancer is a chronic debilitating condition and sometimes needs life-long management.

With the advancement of tumour biology understanding, specific pathway inhibitors with promising result came up. Since the beginning of this century, a large number of drugs with a spectrum of activities are come up. A neoplasm can be the results of atypical proliferation of tissues due to genetic mutations. In spite of all developments in medical field,

cancer a disease as old as manhood is still exists globally as one of the major health problem. Latest reports from the international cancer research agency indicates that approximately 12.8 million new cancer cases and 7.7 million cancer death occurred and also that of these 56% of all new cancer cases and 63% of the deaths due to cancer were in the undeveloped areas of the world. Projections are that by the year 2020, the incidence of cancer will increase by three-fold and that there will be disproportionate rise in cancer related cases and deaths.

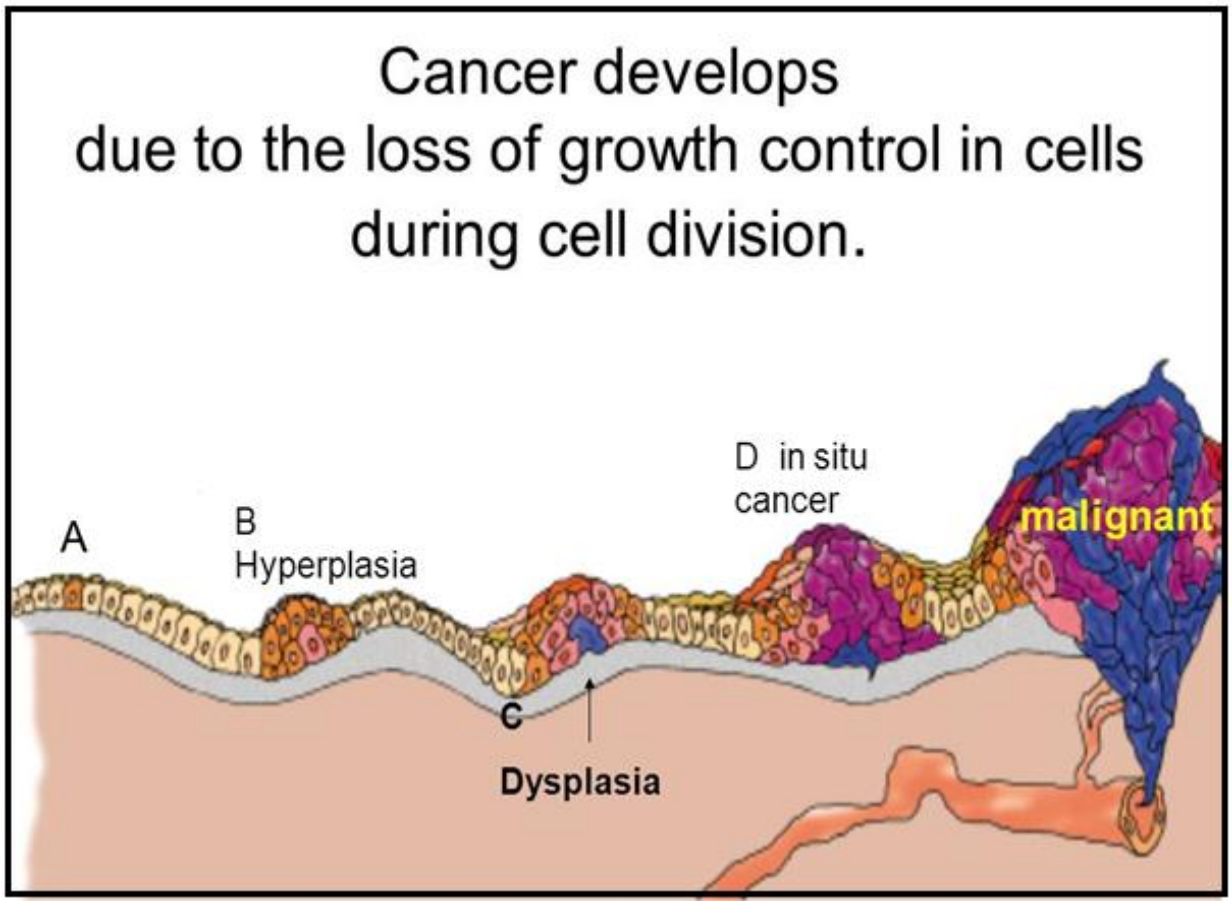


Figure-1: Stages of tumor devolopment

Based to the study conducted by ICMR in 2016 (The Indian Council of Medical Research) believed that, the total number of new cancer cases in India is expected to be about 14.5 lakh and this may likely to reach nearly about 17.3 lakh new cases in 2020. The one-fifth of the world's incidence of cancer cases are expected to be in India. The north-east of India reported the maximum number of cancer cases in both males and females. The Aizawl district in Mizoram state reported the highest number of cancer cases among males in India, whereas

Papumpare district in Arunachal Pradesh state recorded the maximum number of cancer patients among females. "Breast cancer is estimated around 1.5 lakh (over 10% of all cancers) new reports during 2016, is the number one in cancer overall. Lung cancer cases are estimated around 1.14 lakh (83,000 in males and 31,000 in females).

The conventional cancer treatment methods are surgery, radiotherapy and chemotherapy. Chemotherapy is a major treatment method widely used to control the advanced phases of malignancies and as a prophylactic against possible metastasis, shows severe toxic side effects like diarrhoea, fatigue, nausea, vomiting etc. The main objectives of cancer treatment include prolongation of life, cure & relief of various symptoms. Surgical procedures and radiation therapy provide the best treatment for patients with localized cancers; however systemic treatment methods are essential for systemic cancer therapies. Long-term complications of cancer treatment, such as infertility problems, secondary malignancies, effects on physical, mental or intellectual development and vital organ damage, can destructively affect health and quality-health of life for cancer survivors.

Epidemiological studies have consistently shown that proper consumption of fresh fruits and vegetables is strongly associated with reduced risk of developing lifestyle diseases. Cancer is the second leading causes of death in India.1/2 of all the men and 1/3 of all the women will develop cancer during their life-time. Millions of people are living with cardiovascular diseases and cancer. Cardiovascular diseases and cancer are examples for lifestyle diseases. The risks of developing lifestyle disease are reduced by changes in the diet and lifestyle. There has been an increasing appreciation and understanding of the relationship between dietary fruits and vegetable consumption and better-quality health in humans. Research has shown that biologically active constituents in plant-based foods items, mainly phytochemicals, have important potential to modulate many processes in the progress of diseases, such as cancer, CVS diseases, diabetics etc.

It is estimated that 1/3rd of all cancer related deaths could be prevented by improved diet, mostly improved consumption of fresh fruits, whole grains and fresh vegetables. There is undoubted indication that diets high amount of fruits and vegetables are linked with reduce the risk of cancers in the lung, oral cavity, oesophagus, stomach, and colon.

Fruits and vegetable are highly rich in essential nutrients and potent phytochemicals of therapeutically significant. These nutraceuticals components are plant derived chemicals, defined as non-nutritive bioactive compounds derived from natural plant source such as a flavonoids or carotenoid that have been connected to reducing the threats of major life style diseases or considered to have a beneficial effect on human health. They are also called phyto-nutrient or nutraceuticals.

It is estimated that more than 5000 phytochemicals have been separated from fresh fruits and vegetables, but a large quantity still unknown and need to be identified before to understand the health benefits of phytochemicals in food items. But, more and more convincing evidence suggests that the benefits of phyto-constituents in fruits and vegetables may be even countless than is presently understood, because the oxidative stress prompted by free radical molecules play vital role in aetiology of a wide range of chronic diseases. Certain phytochemicals found in the fruits having strong antioxidants activity and are supposed to diminish the risk of chronic disease by free radicle scavenging action, by fluctuating metabolic pathway and detoxification of carcinogenic substances, or even by influencing processes that modify the course of tumour cells.

Many phytochemicals found in fruits and vegetables have an anti-carcinogenic (anti-cancer) action by following mechanism,

- By slowing cell proliferation (division) by interfering with the cell cycle
- By Inducing apoptosis (cell suicide)
- By Inhibiting phase 1 enzymes (enzymes which convert harmless substance into carcinogenic substance)
- By Inducing phase 2 enzymes (enzymes that can attach carcinogens to substance that help immediate excretion).

Phyto-constituents or nutra-ceutical compounds are not classified as vitamins, but they have great contribution to human health and well-being. We are adapted to a world that encloses phytochemicals in our normal diet. The macula of the eye is adapted to concentrate the yellow

carotenoids like zeaxanthin and lutein which help to safeguard against dangerous blue light. However the positive effects of phytochemicals, many can be toxic and harmful in nature.

All the fruits may offer protection to human body against various types of chronic diseases. So that the old proverb defines that "consumption of apple fruit in a day, will keep away the physician from earning his bread". Eating nutrient rich food consumption keeps healthy individual free from disease. While looking for a nutritious food, earlier people believed that, among the fruits, apple are a good place to start. Research over the previous few periods has revealed that apples are really a very healthy snack, According to the American cancer society guidelines on nutrition & physical activity for cancer prevention, American cancer society and world cancer research fund 2015 suggests that eating of a healthy diet, with an emphasis on fruits together with moderate exercise and quitting bad life style fashion may help to prevent carcinogenesis, reduce side effect of chemotherapy and radiation therapy, prevent recurrence and improve quality of life and help to fight many other degenerative diseases of modern era. This may reduce the risk of cancer. Acai berries, Annona, Apple, Avocado, Dragon fruit, Grapes, Orange, Pomegranate, Papaya, Watermelon, Peaches, Strawberries, Chikoo etc. are the anticancer fruits recommended by world cancer research fund 2015. These recommendations do not support the consumption of processed food or nutra-ceuticals, instead require natural conception.

Natural products remain an important source of new drug, by discovery new drug lead moiety or new chemical entities. The plant based drug discovery resulted in the development of many anti-cancer compounds including plants (etoposide, paclitaxel, camptothecin, topotecan vincristine, vinblastine, and irinotecan), resveratrol (grapes, berries and peanuts), ellagic acid (pomegranate), limonene (citrus fruits), ursolic-acid (apple), β-carotene (carrots), and dietary fibre. The active principle compounds derived from natural products are offering a good opportunity to evaluate not only about new chemical category of anti-cancer moiety, but also novel lead moiety and potentially relevant pharmacological action. The anti-proliferative effect of fruits was specific to cancerous cells of origin and was found to be essentially free of their anti-oxidant properties.

LYCOPENE

Lycopene is responsible for the red coloration of tomatoes, watermelon, pink grape-fruit, guava & papaya. The natural trans form is poorly absorbed. The light & heat converts the trans-form to the cis-form, which is more bioavailable. It can reduce LDL cholesterol levels. Act as a powerful antioxidant which reduces damage to DNA and proteins. Having the ability to suppress Insulin-like Growth Factor (IGF-1) stimulation of tumour growth it will concentrates in the testes, adrenal gland, skin, and prostate gland where it it helps to protects from cancer. Exogenous administration of lycopene individual gradual doses in hyper-glycaemic rats causes a dose-dependent reduction in glucose level. Lycopene protects kidney cells from streptozotocin (STZ) induced lesions due to its antioxidant activity by inhibiting NF-κB signalling pathway of anti-inflammatory (Ying Guo et al., 2015)

LIMONENE

This is a sub-class of terpenes, majorly found in citrus fruits, a peel that is specifically directed to protect lung tissue and prevent breast cancer (Kim et al., 2012; Sun et al., 2013). It is 45 times more anti-carcinogenic than hesperetin stimulates cancer cell apoptosis and detoxifies carcinogenic compounds. L-limonene is smells "piney" (like turpentine) and D-limonene smells similar to orange and used as a solvent and cleaner. The main action of limonene is to promote glutathione-S-transferase (detoxification by glutathione addition). In pharmaceuticals industry, limonene is used in medicinal ointments and creams penetration enhancer in the skin.

ANTHOCYANINS

It is a hydrophilic vacuolar pigment that may appear in different colours such as red, purple, or blue based on the pH. They belong to the class of molecules called as flavonoids synthesized via the phenyl propanoid pathway. They are generally odour-less and nearly flavour-less, contributing to taste as a moderately to astringent sensation. Its main action is protects endothelial cells from oxidative damage. These is a powerful compounds may help prevent Coronary vascular diseases and cancer and boost cognitive function (Densie Webb 2014). It is found rich in sapodilla and grapes.

HESPERETIN

It is a flavanone, main bio-flavonoid found in oranges and grapes. Its main action is slows proliferation of cancer cells and slows replication of viruses, including polio, herpes & flu. Herperidin is a derivative of hesperetin (hesperetin 7-rutinoside) is a non-hydrophilic flavonoid glycoside compound its water solubility is less than 5 µg/ml. Hesperidin is found rich in citrus fruits and upon ingestion it releases it's a glycone part, hesperetin (Majumdar S et al., 2009). Hesperidin alone or in combination form with other compounds is used to treat conditions related to blood vessel such as hemorrhoids state ,varicose veins, and poor blood circulation (venous stasis). It is also used to treat lymphedema state, a condition involving fluid retention that can be a complication of breast-cancer surgery. Hesperetin is a cholesterol lowering flavonoid found in citrus juices, may have anti-oxidant, anti-inflammatory, anti-allergic, hypo-lipidemic, vaso-protective and anti-carcinogenic actions.

NARINGIN

It is also a flavanone, gives grapefruit its characteristic bitter taste may cause improve the taste by taste-bud stimulation. It will reduce LDL cholesterol, but not high density lipid cholesterol, may affect with gastro-intestinal enzymes, thereby increasing oral drug absorption. Increases metabolism of alcohol and lipid in the liver, although increasing liver antioxidant activity and protects against radiation-induced DNA damage also having potent anti-apoptotic properties.

QUERCETIN

It is a flavonol, found abundantly in red onions, buckwheat, red grapes, sapota and green tea (highest in apple skins). Its main action includes strong antioxidant, reduces LDL oxidation, in CVS it causes vasodilator and blood thinner. In immune system it causes antihistaminic activity can relieve allergy symptoms and it can kill viruses, such as herpes. It will inhibits COMT enzyme (Catechol-O-MethylTransferase) thus reducing Adrenaline metabolism (increased adrenaline increases fatty acid oxidation and energy expenditure) also having sirtuin like deacetylase action.

PUNICALAGIN

It is found in pomegranate an ellagitannin a type of phenolic compound. It can be metabolized into other compounds (ellagic acid, urolithins). It is ten times the antioxidant potency of ellagic acid and fully water soluble and has high bioavilability, with 95% absorption from the intestine. Main pharmacological action involves suppresses IL-1β inflammatory cytokine Punicalagins are the important constituents in pomegranate juice responsible for its antioxidant property.

CHLOROGENIC ACID

It is associated to esters of OH-Cinnamic acids with quinic acid derivatives. It's found very high amount in blueberries, tomatoes and sapodilla found in the skin and flesh of grapes, along with ellagic acid most frequently an ester of caffeic acid. It may act as a pro-oxidant in the propagation phase of LDL oxidation. Potentially it exerts an anti-diabetic effect. It has also been associated in weight-loss and exerting an anti-obesity effect.

FERULIC ACID

It found abundant in cell walls, seeds of brown rice, whole wheat, apple, orange, and pinapple. It is precursor of vanillin and a good antioxidant anticancer sirtuin and antitumor activity.

RESVERATROL

Resveratrol (RSV) chemically 3, 4', 5-trihydroxytransstibene, is a polyphenol compound most abundantly seen in grapes (Russo et al. 2005) shows the strongest sirtuin like deacetylase property. Sirtuins have been shown to extend the life span of yeast and fruit flies. But, there are some other sources of RSV besides alcoholic beverages (red wine) such as purple grape juice. Resveratrol is a stilbene, a phytoestrogen derivative especially high in grape skin. Its action involves anti-inflammatory, inhibits cyclooxygenase 1 enzyme, inhibits adhesion of blood cells to blood vessel walls, shown to decrease skin and breast cancer in mice, induces phase 2

enzymes, and inhibits NF-κB transcription of pro-inflammatory and cancer-promoting anti-apoptotic genes.

PECTIN

Pectin is soluble fibre in apples will gives feeling of fullness of stomach when eaten. It binds to sugars moiety, releasing them very slowly and keeping blood sugar levels steady and lowers cholesterol.

CAROTENE

The term carotene is used to relate many unsaturated hydrocarbon compounds having the formula $C_{40}H_x$. Fruits are the one of the sources of carotene. Beta-carotene is one of a class of orange, red pigments also act as a precursor of vitamin A, having good antioxidant property, which helps to protect cells and tissues from damage, and act as a essential nutrient. The yellow colour pigments are called carotenoids.. Research studies recommend that dietary consumption of foods high in beta-carotene has positive link with reducing the dangers of CVS disease as well as oral cavity, GIT and lung cancers.

INDOLES

The Indoles are phytonutrients that interact with Vit C and their complexes bind with chemical carcinogens and helps in activating the detoxification enzymes. The acid pH in stomach helps in the formation of metabolized products of indoles like the ascorbigen.

DIETARY FIBERS

Dietary fiber also called as roughage is the in-digestible portion of diet from plants source. The fibers soluble in water will readily fermented in the GIT into gases stage and physiologically active end products, and can act as prebiotic. Research work reports that people who consuming very little amounts of fruit and vegetables have the higher the risk of colorectal cancer. Regular intake of cereal fiber can reduce the risk of colorectal cancers (Paul Terry et al 2001)

The nutritional fact is both a quantity and a quality matter, and fruits in all their various forms safeguard satisfactory intake of most of the essential nutrients and vitamins, dietary fibers, and phytochemicals which can convey a much-needed measure to maintain balance diet habit to resolve many of the nutritional problems. The promotion of healthy fruits products consumption has coincided with a surging interest of consumers in the healthy and dietary functionality of food. Because each fruit comprises a distinctive combination of phytonutriceuticals, a vast diversity of fruits should be eaten in normal diet to get all the health benefits and that to safeguard individuals from life style diseases. In order to maintain a healthy well-being condition have to consume micro-nutrient fortified foods and drink healthy drinks high in vitamins and minerals i.e., more fibrous contain foods with zero sugar and cholesterol. Daily intake food should be balanced with essential nutrients. A balanced diet habit helps to improve the immunity system and keeps healthier. Another very important thing is doing physical exercise every day. The walking is the best and the easiest form of physical exercise. Must go for one hour brisk walking daily. Regular physical exercise supports to burn extra calories & avoids becoming more obese. Adequate and right sleep is also an important aspect for maintaining good health, proper sleeping habit will keep healthy and daily consumption of food should be balanced with essential nutrients. This helps to get the daily quota of vital nutrients. The control and balanced diet habit helps to improve the immunity level and dietary supplements help to provide nourishment but not act as a full substitute to a balanced diet. For this consumption of fruits play an important role.

In view of these it is hypothesized that a combination of fruits adequately supplying required nutrient in excess may have effect of pharmacologically relevant natural constituents it contain that promote human health and resist diseases. The combo of fresh fruits formulation that can supply adequate amount nutrients as well as nutraceutical with different therapeutic effects in healing of various diseases.

In the study, we designed a combination of five different fruits providing different phytonutrients required for the human body to maintain RDA of essential nutrients. Orange, grape, sapota, apple and pomegranate possess many natural substances that appear to be important in disease protection. Together these phytochemicals may act

synergistically/additively. In view of these the protective effect of fruits combo has been investigated against cyclophosphamide induced toxicity using swiss albino mice through improving antioxidant status.

2. LITERATURE REVIEW.

Cancer results from a sequence of molecular events that fundamentally modify the normal properties of cells. During cancer there may be uncontrolled proliferation of cell in which, cancer cells that cause normal control systems that put a stop to cell overgrowth and the attack of other tissues are disabled. While it is true that the tumors that forms spontaneously in our bodies, usually microscopic size, posing no risk or hazard to health, it is also true that, all these tumor cells do not grow and develop into lethal end stage cancer. However, if the cell does grow, there will be at possibility of developing cancer. Anti-cancer molecules present in fruits and vegetables flight against cancers at the source, before it can reach at maturity. Anti-cancer molecules present in food especially in fruits and vegetables keeps them from progressing to a progressive phase of death. So cancer is a chronic disease, one that can be maintained on a day-to-day basis with the help of diet rich in anti-cancer compounds. Among the many anti-cancer compounds present in fresh fruits and vegetables, the phytochemicals are the important. Current scenario communicable diseases like tuberculosis, malaria, typhoid will become less significant foundations of fatality. Modern science through enhanced sanitation, vaccination, antibiotics therapy, and therapeutic consideration has eliminated the danger risk of death from utmost infectious diseases. Diseases like cardiovascular disease, cancer, stroke, diabetics and traffic accidents are claim larger percentage of sufferers (Mcneil et al., 2008)

According to INTERNATIONAL AGENCY FOR RESEARCH ON CANCER, WHO (2014) WORLD CANCER REPORT 2014 tells that non-communicable diseases (NCDs), also known as chronic diseases or lifestyle disease, are not passed from person to person. The natural way of the relationship between diet style and disease is too complex. Increasingly, nowadays research studies has been encouraged to studying identification of lead moiety from plants, vegetables and fruits to achieve a greater understanding of their exact role or mechanism of action involved in the prevention and cure or decrease of disease in humans beings.

PHARMACOLOGY OF CYCLOPHOSPHAMIDE (CTX)

Cyclophosphamide is alkylating agent, belong to nitrogen mustard (sub-class of anti-cancer drug) (Milsted AVR, et al., 1982) act as an immuno-suppressive and cytotoxic agent

used in various medical problems. In human serum the half-life of cyclophosphamide is about 6.5 hours. Tubular reabsorption of cyclophosphamide (CTX) is very high. So, a very small fraction of it excreted from kidneys (Gershwin ME et al., 1974)

The initial step of activation of cyclophosphamide (CTX) includes hydroxylation and forms 4- hydroxyl-cyclophosphamide. This metabolite breaks into two cytotoxic metabolites named as acrolein (toxic) and phosphoramide mustard. Cyclophosphamide enzymatically metabolized into 4-hydroxycyclophosphamide (Hales FB 1982), which exists in equilibrium with aldo-phosphamide as shown in (fig 2). By the help of aldehyde dehydrogenase enzyme, most of the aldophosphamide oxidized and convert into carboxy-phosphamide. And a small portion of aldo-phosphamide convertes into phosphoramide mustard moiety and highly toxic species (acrolein). This acrolein is toxic for the epithelium of bladder and to hepatocytes. Inactive urinary excretory metabolic products like 4-ketocyclophosphamide and carboxy-phosphamide are also forms during phase-II biotransformation of cyclophosphamide. Phosphoramide mostly cycles into the quaternary aziridinium ion and alkylated the DNA molecules (Chen TL, et al., 1997). Acrolein is an electrophilic in nature. It is the highly reactive aldehyde moiety causes extensive damages the cells of the kidney and urinary bladder (Lemke LT et al 2008)

ANTI-CANCER EFFECTS OF CYCLOPHOSPHAMIDE (CTX)

Colleonii et al., 2001 described the effect of oral administration of methotrexate and cyclophosphamide (CTX) at low dose in metastatic stage of breast cancer in women and anti-tumor property relationship with vascular endothelial growth factor levels (VEGF). They assessed the clinical efficacy by means of direct cytotoxicity on tumor cells. The continuous low dose cyclophosphamide and methotrexate is minimally toxic and effective in pretreated breast cancer patients and there is a reduction in the VEGF which was related with the treatment. From this they found that Combination treatment that prevents angiogenesis and cytotoxic chemotherapy may be effective than either type of therapy alone.

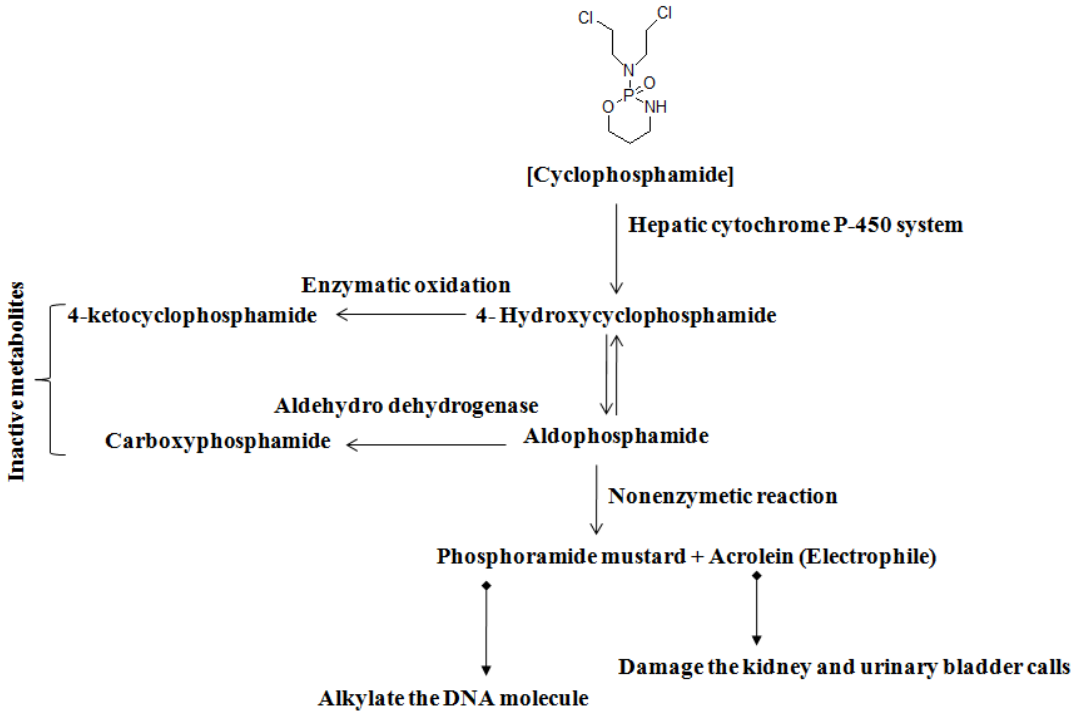


Figure 2: Metabolism of Cyclophosphamide.

Touil et al., 2011 carried out a study on anti-angiogenic and anti-tumour activity of the combination of the natural flavonoid compound, fisetin and cyclophosphamide (CTX) in Lewis lung carcinoma-bearing mice. The antioxidant Fisetin was administered i.p. at 223 mg/kg daily for five days in the first week (days 4 to 8 post tumour implantation) and followed by 3 injections on 11^{th}, 12^{th} and 14^{th} day. Fisetin treatment controlled to a 67% tumour growth reduction compared to the controls. The low dose cyclophosphamide was administered at 30 mg/kg on 4 days in a week 1 only (days 4, 5, 7, 8), and led to a tumor growth inhibition of 66%, similar to fisetin treatment. The same dose schedule of combined fisetin and cyclophosphamide causes the reduction of tumor volume dramatically ie 92% inhibition when compared to controls on day 15. Over the 2 week treatment, only a 4.6% loss in body weight was observed showing that drug combination was not toxic.

IMPORTANCE OF FRUITS CONSUMPTION

T J Key 2010 stated that cancer risks and consumption of fresh fruits and vegetables, recommended that healthy diet should include at-least enough amount of fresh fruits and vegetables, adequate amount of fresh fruits and vegetables consumption prevent deficiencies of nutrients especially Vitamin C, which are mostly supplied by fresh fruits and vegetables. The increases in fresh fruit and vegetable consumption would not have much effect on cancer rates.

Hertog MG et al., 1996 investigated the significance of consumption of fruit and vegetables and chances for lowering cancer mortality in a cohort of 2112 Welsh men ages at range of 45-69 years. Participants allowed to having Mean intake of vegetables and fruits at baseline were 118 gram/day and 83 gram/day respectively. Related to all-cause cancer-mortality and the strongest association were witnessed for fruit consumption and its importance in reducing the cancer mortality rate. The regular Consumption of vegetables and predominantly the consumption of fresh fruits (apple, orange, cherry, pomegranate, straw berries, annona etc. could significantly reduce the risk of dying from cancer in middle aged men.

ROLE OF NUTRACEUTICALS IN HUMAN HEALTH

Dillard et al., 2000 reported that phytochemical are compounds made by plant derived orgin that contain various nutrients such as vitamins, minerals, and antioxidants that impart a great effect in the body. The majority of the phytochemicals are known as antioxidants, carotenoids, beta-carotene, folic acid, vitamin C, and vitamin E. phytochemical could provide health benefits such as

- Substrate for the biochemical reaction
- Co-factors for the enzymatic reaction
- Inhibitors for the enzymatic reaction
- Absorbents or sequestrates that bind to form complex to eliminate undesirable compounds in the GIT
- Scavengers of the toxic substances

- Ligand that act as agonist or antagonist on cell surface or intracellular receptors
- Compounds that enhance the absorption and stability of essential nutrients
- Selective growth factors for the beneficial GI bacteria
- Fermentation sources for useful intestinal flora
- Selective inhibitors of deleterious intestinal bacteria. The `new' nutraceuticals of plant origin may evolve to be considered a dynamic characteristic of nutritive disease-preventive food components.

Rahimi et al. 2015 reported that curcumin is a nutraceutical from the turmeric can act as hepato-protective. It has been found that curcumin can prevent gene expression, causing in decrease the sensitivity of TNF-α, interleukin 1 (IL-1), IL-6, IL-8 and C-reactive protein. Hence, Curcumin can have good therapeutic effects on the various chronic diseases.

Surh YJ 2003 reported that chemo-prevention refers the use of agents to inhibits, reverse or retard tumour genesis. Numerous nutraceuticals derived from edible plants sources have been specified to interfere with a specific stage of carcinogenic process. Many mechanisms have been revealed to description for the anti-carcinogenic property of dietary constituents, but the attention has recently been focused on molecular targets for several pharmacologically potent phytochemicals.

FRUITS DERIVED THERAPEUTIC AGENTS

Kasdallah-Grissa et al., 2007 reported the effect of RSV on LPO and antioxidant enzyme level status in the brain tissue of healthy rats. Resveratrol, a natural phytoalexin present in grape skin holds a diversity of biological activities including antioxidant property. Dietary supplementation with resveratrol (RSV) during ethanol administration prevents hepatic lipid peroxidation and improved SOD, GPx and CAT level in the liver. The i.p. administration of resveratrol causes dose dependent reduced brain malondialdehyde. Increased in a dose-dependent way brain superoxide dismutase (SOD), catalase (CAT) and peroxidase activities can cause by resveratrol (RSV). Optimal antioxidant and LPO products pharmacological effect was found with resveratrol concentration of 12.5 mg/kg body wt. The highly lipophillic blood brain barrier (BBB) were easily cross by resveratrol and exerts potent antioxidant activity in brain.

Ying Guo et al., 2015 assessed the protective effect of lycopene, a powerful antioxidant which reduce the damage to DNA and Protein and dose-dependent reduction in blood glucose level was found at administration of lycopene to hyper-glycaemic rats. Due to lycopene antioxidant property helps to protects kidney cells from streptozotocin (STZ) induced lesions by inhibiting NF-κB signalling pathway of anti-inflammatory and attenuating oxidative stress for anti-dysmetabolism.

Majumdar S et al., 2009 studied the significance of hesperidin (obtained from citrus fruits), a derivative of hesperedin is chemically it is hesperetin 7-rutinoside is a non-hydrophilic flavonoid glycoside compound its water solubility is less than 5 µg/ml. Hesperidin is found rich in citrus fruits and upon ingestion it releases its a-glycone part, hesperetin. Hesperidin alone or in combination form with other compounds is used to treat conditions related to blood vessel such as hemorrhoids state ,varicose veins, and poor blood circulation (venous stasis)

IMPORTANCE OF FRUITS CONSUMPTION

The effective, promotion of fruits consumption can significantly contribute to a well-balanced diet. This significance was outlined and highlighted also in the World Health Organization Technical Reports entitled "Nutrition, diet, control and prevention of various chronic diseases" published 2003. Many authors go into the detail data of this topic, among them Boeing H et al. 2012 report on a convincing evidence of the reducing risk for cardiovascular disease and a possible evidence for cancer, overweight, type 2 diabetes mellitus and some other disorders as a result of increased fruit and vegetables consumption. Various nutrients are consumed via, the food that we eat, and through metabolic processes in the gastro-intestinal tract these nutrients are absorbed at small intestine to cellular level in the body and Optimum nutrients level in the body contribute to good-health, wellbeing, normal development, and high quality of life. But, under-nutrition, over-nutrition, and malnutrition are connected to suboptimal health issues Gibney et al., 2009. Such a type of poor diet conditions have been linked to the incidence of various chronic diseases, such as cardiovascular disease (CVS), Type-II diabetes, cancer etc Lytle et al., 2002. A perusal of published literature manifests that the subject of consumption of low intake of fruit and vegetables higher the risk for developing cancer (Steinmetz et al., 1996), as well as CVS disease Hungg et al., 2004, while lower the consumption of dietary fibres has been related to being overweight Patrick et al., 2004.

The screening of various literatures reveal that research work has been published dealing with the Fruit. Reports related to Cancer, and Cardiovascular Disease Mortality by Genkinger et al., 2004 acknowledge the contributions that higher intake of fruit and vegetables may help protect against oxidative damage, thus lowering cancer and cardiovascular disease risk reports that, antioxidant nutrients were hypothesized to decrease risk of all-cause, cancer, and cardiovascular disease mortality.

A comprehensive account of the role of fruit consumption in the prevention of obesity has been published by Tetens et al., 2009. The survey of literature shows that selected fruits and fruit-products (apple, orange etc.) Of low energy density and relatively high dietary fiber contents have been shown to improve postprandial satiety and consequent lower hunger in subjects of normal weight. Based on their data evidence they concluded that fruit consumption has an important role in the prevention of overweight related problems and obesity.

APPLE

The significance of apple fruit consumption is well known that while evaluating the reports of various writers. The many phytochemicals found in apple have been shown to be biologically potent and may interact to protect against various cancer. Experimental research have provided indication for important useful effects of flavonoids on several cancer related biological pathways such as carcinogen bio-activation, cell-signaling pathway, cell-cycle regulation, angiogenesis process, oxidative stress and inflammatory pathways (Le Marchand et al.,2002)

Boyer and Liu 2004 reported about phytochemical present in apple and their major health benefits and found that storage has little to no effect on apple phytoconstituents, the strong antioxidant components present in apple are Quercetin, Catechin, Phloridzin and Chlorogenic acid etc.

Le Marchand et al., 2000 their review included an overview of the positive association between apple products and health benefits. Research suggests that consumption of flavonoids rich foods may safeguard against lung cancer and that decreased bio-activation of cancer causing agents by inhibition of CYP1A1 metabolic enzyme.

➢ Kingdom	Plantae
➢ Division	Magnoliophyta
➢ Class	Magnoliopsida
➢ Order	Rosales
➢ Family	Rosaceae
➢ Genus	Malus
➢ Species	M. domestica

Table 1: Botanical classification of Apple

Jensen et al., 2009 reported that Apples are beneficial supplement to a heart-friendly diet. Mechanism behind the hypo-lipideamia effect of apple may involve an increased clearance of plasma cholesterol due to greater fecal excretion of bile acids and cholesterol. The filtered apple juice has low activity compared to apple fruit due to lack of fibers and increased sugar content.

Gallus et al., 2005 has indicated that compared with other normally consumed fruits, apples had the second highest level of antioxidant property (after cranberries). Their research found a reliable inverse relationship between apples fruit consumption and risk of various cancers such as pharynx, esophagus, colorectal, breast, ovary etc..

GRAPES

The antioxidant properties of phenolic compounds present in grape have been widely studied in vitro and in vivo. Many scientists stated that grape skin, seed, and pomace extracts have potent free radical scavenging activities using oxygen radical absorbance capacity. But, the in vivo studies examining anti-oxidant activity of grape extracts have revealed unreliable results. Some studies showed that dietary consumption of grape anti-oxidants helps to prevent lipid oxidation and inhibit the formation of ROS. Further, the anti-oxidant potency of grape is proved by many scientists. Resveratrol a grape phyto-constituent has drawn greater attention because of its valuable effects as anti-cancer phyto-constituent (Signorelli P et al., 2005). However, Resveratrol (RSV) has some demerits such as un-stabilization (Signorelli P et al., 2005)., less water solubility (Lu Z, et al., 2009) and short biological half time ($t_{1/2}$) (Juan ME, et

al., 2002), which limit the utilization of Resveratrol in medicine, food and pharmaceutical industries.

➢ Kingdom	Plantae
➢ Division	Magnoliophyta
➢ Class	Magnoliopsida
➢ Order	Vitales
➢ Family	Vitaceae
➢ Genus	Vitis
➢ Species	V.vinifera

Table 2: Botanical classification of Grapes

Jonathan et al 2012 was studied the effect of grapes (vitis vinifera) aqueous extracts on Gram positive (G +Ve) and Gram negative bacteria (G -Ve) and their antioxidant property. The Grapes are considered as a richest source of poly-phenolic compounds that show potent antioxidant and good antimicrobial activities. The anti-oxidant potency of the grapes depend on the content of total phenol compounds, and the antioxidant status and anti-bacterial activity were found to be lower when extracts were obtained from extracts after production of wine. The results recommend that the use of grape extract is a feasible substitute as antibacterial and antioxidant agents to avoid the deterioration of stored food-stuffs by bacteria and oxidation.

Yilmaz and Toledo 2004 reported that seeds, pulp and skins of grapes are very worthy sources of phyto-chemicals like catechin, epi-catechin and gallic acid and are appropriate raw materials for the production of anti-oxidative dietary supplements. The differences in stages of the major monomeric flavanols and phenolic acids in seeds and skins from grapes of Vitis vinifera varieties and in seeds from grapes of Vitis rotundifolia variety muscadine were evaluated for antioxidant properties. The influence of the major monomeric flavonols and phenolic components of seeds and skins of grape to the total antioxidant property was also determined. Gallic acid, monomeric catechin, and epi-catechin concentrations were found 99, 12, and 96 mg/100gm of dry matter in Muscadine seeds, 15, 358, and 421 mg/100gm of dm in

Chardonnay grape seeds. Concentrations of these three compounds were lesser in winery by-product of skin of grapes than in grapes seeds. These 3 chief phenolic compounds of seeds of grape contributed catechin > epicatechin = gallocatechin > gallic acid = ellagic acid. The results showed that most of the superior antioxidant capacity of grape seeds is due to dimeric, trimeric, oligomeric, or polymeric pro-cyanidins.

Baydar et al., 2011 reported anti-oxidant activity of Grape extract done by different solvents. They reported total phenolic contents of each extracts by Folin-Ciocalteu method. Antioxidant properties of the grape extracts at various concentrations were estimated using the DPPH radical scavenging, hydrogen peroxide scavenging and phosphormolybdenum methods. Likewise the extracts, as natural antioxidants compounds, were assayed throughout 8 weeks storage of refined poppy oil at 70 °C. By determining grapes extract capability to scavenge DPPH and H_2O_2 showed strong antioxidant property; to reduce Mo (VI) to Mo (V) and peroxide formation rate will be inhibited, while compared with the bagasse extracts. The grape extracts antioxidant activity was found increase with increase concentration of extract increases

ORANGE

➢ Kingdom	Plantae
➢ Division	Magnoliophyta
➢ Class	Magnoliopsida
➢ Order	Sapindales
➢ Family	Rutaceae
➢ Genus	Citrus
➢ Species	C. sinensis

Table 3: Botanical classification of Orange

Guarnieri S et al., 2013 reported that consuming Vit C supplements will not offer the same health benefits as drinking a glass of orange juice. Their study demonstrates both antioxidant property and cytoprotective property that reflect their significance role in preventing severe pathological conditions.

Entezari et al., 2008, reported the anticancer and anti-mutagenesis effects of Citrus Fruit Juice. They perform Ames test to consider its anti-cancer effect highlights on application of Gram negative bacteria salmonella typhimurium to determine anti-mutagenesis and anticancer level of chemical substances. In this research, half-ripe and ripe fruit juice shown anticancer and anti-mutagenesis effect, in which half-ripe fruit juice was highly effective than ripe fruit juice. In-vitro, study on fruit juice effects on cancerous cell culture shown that the fruit juice strictly repressed division of cancerous cells, which in this work the effect of half-ripe citrus medica fruit juice was higher than ripe one.

Silalahi 2013 described the protecting effects of citrus fruit constituent emphasis to cancer prevention. Bioactive phytoconstituents present in citrus fruits that are involved in inhibition of various degenerative diseases like, vitamin C, β-carotene, flavonoids, limonoids, folic-acid and dietary fibres. Vitamin C, flavonoids and β-carotene are the main potential antioxidants protective effect against oxidation of molecular biomolecules (DNA, Protein etc) thereby reducing the risk of various lifestyle diseases. The compound limonoid may fight against a various cancers by inducing GST activity to neutralize cancer causing free radical substances.

Riso p et al., 2005 reported antioxidant bioavailability and on various markers associated to oxidative stress on the blood related to orange juice intake. Study was conducted in Sixteen healthy female volunteers were given 600 mL/day orange juice and diet without orange juice for 21 days. They found that orange juice consumption causes significant rise in plasma concentration of Vit-C, beta-cryptoxanthin, cyanidin-3-glucoside, and zeaxanthin.

Duda et al., 2011 reported Antioxidant properties of various fruits seeds and peels. Seeds and peels of commercially available domestic and imported fruits were used in the investigational studies. Among the fruits' parts examined, the maximum antioxidant property was in the peels of the Sampion cultivar and white grapes, and the seeds of the Idared cultivar and orange.

POMEGRANATE

Pomegranate is the fresh fruits obtained from the plant Punica granatum belonging to family-Punicaceae, used as traditional medicine of several nations especially in the Middle-East. The phenolics compounds, tannins, anthocynins flavonoids are the main imoportant phytoconstituents Pomegranate fruits (de Nigris et al., 2007; Ricci et al., 2006). It is commonly called as Annarr in Hindi, Annarmitha in Urdu, Daliimba in Marathi, Dalim in Assamese and Bengali, Dalimbo in Oriya, Dadamm in Gujarati, and Dadimba in Telugu, Daliimba in Kannada and Marathi, Maatalam in Malayalam, Maadulam in Tamil.

Pomegranate is widely reported that pomegranate exhibits anti-viral, anti-oxidant, anti-diabetic, anti-diarrheal, anti-cancer and anti-proliferative activities (Faria et al., 2006; Abdel Moneim, 2012). Pomegranate is an important source of phytochemicals such as tannins, punicalagin, anthocyanins and punicalin (Afaq et al., (2005), gallic and ellagic acids and VitC (Turk et al., 2008). The properties such as antioxidant and free radical scavenging property of pomegranate phenolic compounds (Rosenblat et al., 2006) and vitamins C (Sonmez et al., 2005) have been reported.

N D KIM et al., 2002 reported hemo-protective and adjuvant therapeutic potential of pomegranate (Punica granatum) for breast cancer in women's. The crude whole oil and fresh and fermented juice concentrate of pomegranate were assessed in-vitro for possible chemo-protective effects in breast cancer conditions. The ability to cause a blockade of endogenous active biosynthesis of estrogen was shown by phenolics presentin fermented juice, pericarp, and oil, which is having the ability to inhibit 60–80% aromatase activity.

Chidambara et al., 2002 described anti-oxidant property methanolic pomegranate fruit extracts. This extract of pomegranate at 50mg/kg equivalents to catechin followed by CCl_4 treatment causes preservation of antioxidant enzyme to values comparable with control values, whereas LPO was significantly reduced 54 percent as compared to control. The observations of histopathological data of liver were also carried out to determine the hepato-protection effect exhibited by the pomegranate fruit extract against the toxic effects of CCl_4.

➢ Kingdom	Plantae
➢ Division	Magnoliophyta
➢ Class	Magnoliopsida
➢ Order	Myrtales
➢ Family	Lythraceae
➢ Genus	Punica
➢ Species	P. granatum

Table 4: Botanical classification of Pomegranate

Sharma et al., 1955 reported idea regarding relationships of the chemical content of pomegranate and their desirable pharmacological properties the most common ingredients of pomegranate include anthocyanins, pentose glycosides of malvidiine and pentunidiin have been reported their presence in the pericarp and juice.

Kim et al., 2003 evaluated the total phenolic matters of Pomegranate juice and peel extract then quality were assessed by determining the total phenolic content and assessing the alterations after two and three days of exposure to the same conditions as the juice supplied to the animals in their experimental study. The total phenolic contents of the pomegranate juice and peel extract were 74.8 μg and 124.3 μg gallic acid equivalent/ml juice, respectively, were determined by Folin reagent method. This parameter was not changed for the evaluated period.

Surinder K et al., 2007 evaluated anti-hepatotoxic property of pomegranate acetone extract against isoniazid (INH) and Rifampin Induced hepatotoxicity. This study has reported the effect of Punica granatum fruits 70 percent acetone extracts on liver marker, anti-oxidants, and LPO effect during INH and rifampin-induced hepatotoxicity screening. These reports explained the hepatoprotective property of punica granatum fruits 70 percent acetone extract on tissue defense systems during INH and rifampin -induced hepatotoxicity in rats.

Angel et al., 2007 assessed antiviral effects of whole fruit extracts of plant punica granatum. These aqueous or hydro-alcoholic extracts have proved highly active against the influenza-virus. But, the toxic characteristics of this fruit extract have also been reported. In their study, the genotoxicity of this whole fruit extract was also determined using different in-vitro and in-vivo methods that identify damages to DNA at different expression levels. The result obtained from their study clearly showed that punica granatum hydro alcoholic whole fruits extract is genotoxicity protective effect when tested both in-vitro and in vivo.

SAPODILLA

Sapodilla fruits are egg-shaped or round shape 2-5 inches in diameter. The skin of fruit is brown and scruffy when it ripens. The flesh will varies from yellow to brown color, has smooth or a granular texture. it is obtained from the fresh and ripens fruits of Manilkara zapota, which belongs to the family Sapotaceae, is an evergreen, glabrous tree that is grow throughout the Bangladesh, Indian subcontinent including (Ghani, 2003), Tamilnadu, Andrapradesh, Gujrath & Kerala. The chief phytoconstituents isolated from Manilkara zapota fruits are poly-phenolic compound methyl chlorogenate, dihydromyricetrin, quercitin, myricitin, catechin, epicatechin, gallocatechin, and gallic acid (Ma et al., 2003). Sapodilla is a flock medicine been used in the treatment of communicable diseases, oxidative stress, but there is no data available to validate scientifically related to its uses as the plant yet has not been undergone any extensive chemical or pharmacological study. The concentration of constituents in the sapodilla varies in leaf, fruits, latex, seed and bark.

Sapodilla fruits are egg-shaped or round shape 2-5 inches in diameter. The skin of fruit is brown and scruffy when it ripens. The flesh will varies from yellow to brown color, has smooth or a granular texture. High latex content is found in the raw fruits and bit latex remains in ripe fruit. Ripe fruits id smooth in touch and unripe fruit is rough and leathery to touch. The tannin content of unripe fruit is high compare to ripe fruit is more deliciously more sweet and pleasant. Sapodilla shows wide variety of pharmacological activities such as anti-bacterial (Osman et al., 2011), anti-fungal (Osman et al., 2011), anti-tumor activity (Rashid et al., 2014)., anti-pyretic and anti-inflammatory (Ganguly et al., 2013) (Hossain M H et al.,2012), Hypo-cholesterolemic (Sarada et al., 2014), Anti-diarrhoeal (Manirujjaman et al., 2013), Tyrosinase

and elastase inhibitor effect (Rao GV et al., 2014 and analgesic activity (Munirujjaman sultana F et al., 2014). This fruits is consumed in various forms either as whole fruit or ice creams, fruits salad, fruit shakes etc. sapodilla fruits are considered as a natural energy booster as it contains fructose and sucrose. Sapodilla plant has a various medicinal and cosmetic properties.

➢ Kingdom	Plantae
➢ Division	Magnoliophyta
➢ Class	Magnoliopsida
➢ Order	Ericales
➢ Family	Sapotaceae
➢ Genus	Manilkara
➢ Species	M .zapota

Table 5: Botanical classification of Sapodilla

Osaman et al., 2011 and Sakala et al., 2013 reported the antimicrobial effect of extract of bark and root of sapodilla against gram positive and gram negative bacteria. This shown that wide spectrum antimicrobial property of extract and this property are due to presence of tannins, glycosides, alkaloids, and carboxylic acid in the plants.

Edward J. Kennelly et al., 2003 reported the antioxidant property of manilkara zapota, resulted in the identification and isolation of two new anti-oxidant components methyl-4-O-galloylchlorogenate and galloyl chlorogenic-acid together with 8 known poly-phenolic anti-oxidants. Methyl 4-O-galloylchlorogenate shown the highest value of antioxidant property in the DPPH free radical assay with IC_{50} value was found at concentration of 12.9 mM. The study results of in-vitro cytotoxicity effects of sapodilla in the HCT-116 and SW-480 human colorectal cancer cell lines with IC_{50} values of 190 μM and 160 μM, respectively.

Table 6: **Phytoconstituents of Chikoo**

SL.No	Phytoconstituents	Plant part
1	**ENZYME:** Polyphenol oxidase	Fruits
2.	**PHENOLIC COMPOUNDS (Mathew et al., 1969)** ➢ methyl-chlorogenate, ➢ dihydromyricetin, ➢ quercitrin, ➢ myricitrin, ➢ (+)-catechin, ➢ (–)-epicatechin, ➢ (+)-gallocatechin, ➢ gallic acid	Fruits Leaf Bark
3.	**Ascorbic acid**	Leaf, Fruits and Bark
4.	**Alkaloids (Saradha et al., 2014)** Sapotinine and Sapotin	Whole plant
5.	**Saponin** ➢ Manilkoraside	Stem bark
6.	Beta carotene	Fruit
7.	**MINERALS** Fe, Cu, Zn, Ca, and K	Fruit

3. AIM AND OBJECTIVES

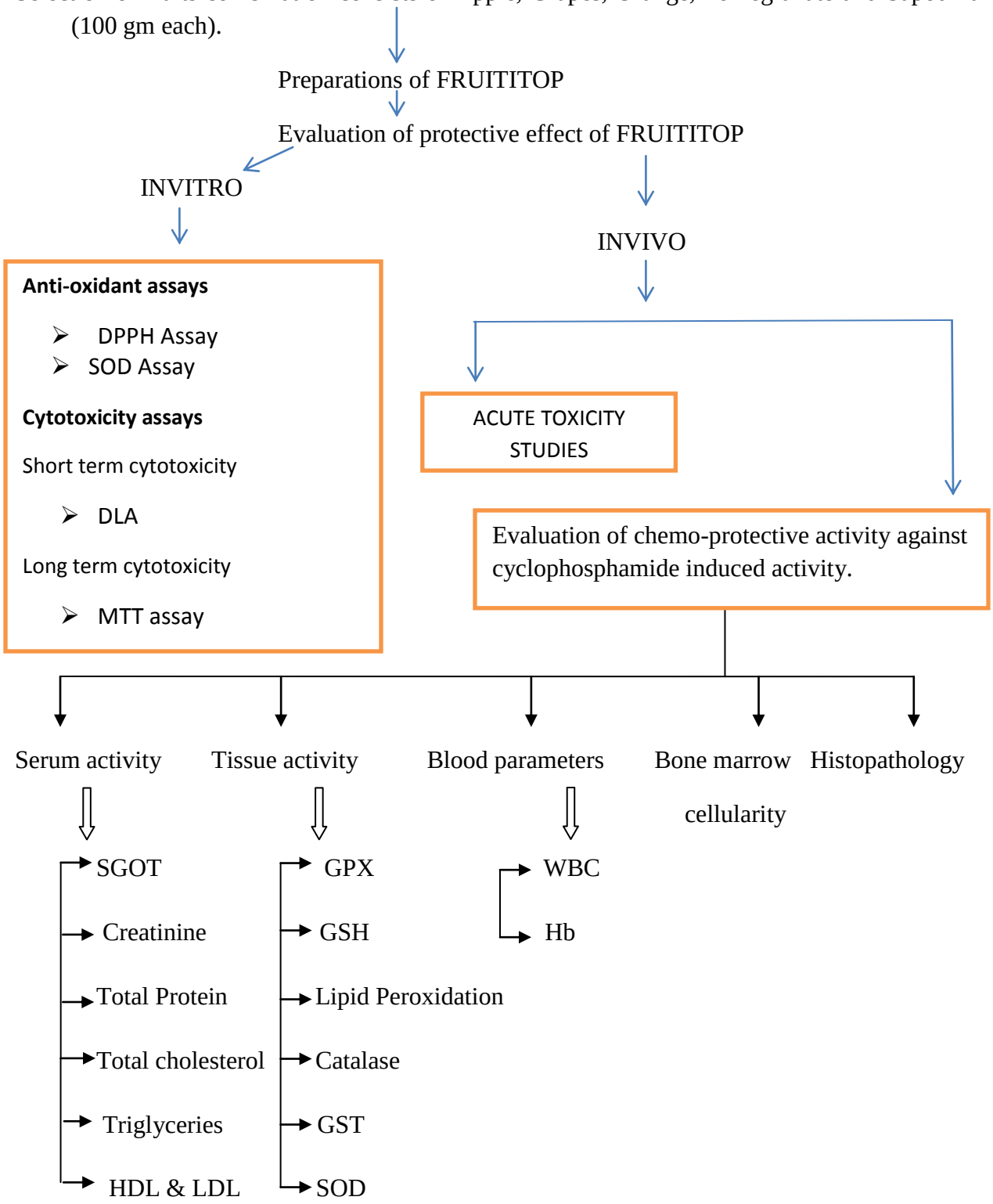
Overall aim of the present work was to evaluate the protective effects of natural fruits combination against cyclophosphamide induced toxicity.

The proper and regular consumption of vegetables and fruits have promising role in prevention and cure of various human diseases as well as restorative to normal health that prevent adverse effect of many medicines. Fruits are rich in vitamins, minerals and various phytonutrients. The selection of combination of fruits was made on the basis of its:

- Consumption of combination of fruits maintains the recommended daily allowance (RDA) of various nutrients required for our body, preventing malnutrition.
- Combination of fruits contains various nutraceutical and adequately supply required nutrient in excess may have synergistic effect of pharmacologically relevant natural constituents thereby promote human health and resist diseases.
- Degree of research work which is not yet performed using combination of fruits.

4. PLAN OF WORK

Selection of fruits combination consists of Apple, Grapes, Orange, Pomegranate and Sapodilla (100 gm each).



5. MATERIALS AND METHODS

The experiments were carried out in the Department of Biochemistry, Amala Cancer Research Institute Thrissur, Kerala.

A- MATERIALS

A.1. REAGENTS AND CHEMICALS

Diphenyl picryl hydrazine (DPPH), Ethylene di-amine tetra acetic acid (EDTA), 2,4-dinitrophenylhydrazine(DNPH), 5,5'-dithiobis-2-nitrobenzoic acid (DTNB),Chromotropic acid were procured from **Sigma-Aldrich, India**. Folin–Ciocalteu reagent, gauanidine hydrochloride, Nitro blue tetrazolium (NBT), H_2O_2, potassium cyanide, reduced glutathione, sodium azide were purchased from **Merck ,India.** Sodium dodecyl sulfate (SDS), Perchloric acid was obtained from SRL Chemicals, Mumbai and Thio-barbituric acid (TBA) from **Himedia Labs, Mumbai.** All other chemicals and solvents used in this work were of analytical grade. Deionized water was used throughout the experiment process. The fruit used are fresh and hygienic were bought from fruits stall in Amala Hospital fruits stall, Amala Nagar, Thrissur, Kerala.

A.2. EXPERIMENTAL ANIMALS

The investigation was undertaken in Female Swiss albino mice of average weight 25–30gm. The mice were purchased from Small Animal Breeding Station, Kerala Veterinary and Animal Science University, Mannuthy, Kerala, India, which was maintained under standard environmental conditions (22-28^{0}C, 60-70% relative humidity, 12hr dark/light cycle) and fed with standard rat feed (Lipton India) and water ad libitum. All animal experiments were conducted during the present study got prior permission from Institutional Animal Ethics Committee (IAEC approved) and followed the guidelines of IAEC.(No:ACRC/IAEC/2016/06-03)

A.3. "FRUITITOP" Formulation and Procurement

The "FRUITITOP" natural fresh fruits formulation was formulated by the following ingredients.

BOTANICAL NAME	QUANTITY (grams)
Malus domestica	100
Vitis vinifera	100
Citrus sinensis	100
Punica granatum	100
Manilkara zapota	100

Table No- 7 Composition of FRUITITOP

A.4. PREPARATION OF FRUITITOP

The fresh Apple (Malus domestica), Grapes (Vitis vinifera), Orange (Citrus sinensis), Pomegranate (Punica granatum) and Sapodilla (Manilkara zapota) fruits, free of blemishes or obvious defects, purchased from AMALA HOSPITAL FRUITS STALL (Amala Nagar, Thrissur, kerala) were washed thoroughly by using deionized water. From this fruits only edible portion was taken for the preparation of formulation FRUITITOP. Each fruits were cut in to small size by sterile stainless steel knife. 100 mg of each fruits were separately weighed. Juice pulp uniform size was prepared by using a commercial blender water was not added, and around one to two hours has taken to make this formulation. Formulation was stored at -80ºC batch wise in a small air tight container used for further studies.

The formulations were subjected to various studies for which the materials and methods presented below.

B. METHODS

B.1.ORGANOLEPTIC EVALUATION

This was used for identification of sensory characteristics like color, odor, taste, texture etc of natural fruit formulation.

B.2. PRELIMINARY PHYTOCHEMICAL EVALUATION

The natural products from plant source such as fruits and vegetables may be considered as a bio-synthetic laboratory, not only for the chemical compounds such as carbohydrates, proteins and lipids that are utilized as food by man but also for a multitude of compounds like glycosides, alkaloids, volatile oils, tannins etc. that exerts a physiologic and therapeutic effect. The compounds that are responsible for medicinal property of the drug are usually secondary metabolites. A systemic study of a crude drug embraces, thorough consideration of primary and secondary metabolites derived as a result of plant metabolism. The pharmacological effects of some of the phytochemical constituents are: Glycosides the organic compounds derived from plants are genetically as a cardio-tonic agents, diuretics and sedatives. Tannins are secondary metabolites which are used in the treatment of diarrhea, hyperglycemia, hypertension etc. Alkaloids are chemically heterogeneous group of natural substances generally used as anticholinergic, local anesthetics and anti-malarial agents.

The formulation FRUITITOP was subjected to qualitative tests for identification of various plant constituents. The phyto-constituents present were shown in Table.10.

Test for Alkaloids		
TEST	PROCEDURE	OBSERVATION
Dragendroff's test	To the pulp, add 1 ml of Dragendroff's reagent	orange red colored precipitate indicates the presence of alkaloids
Wagner's test	To the pulp, add few drops of Wagner's reagent	Reddish brown colored precipitate indicates the presence of alkaloids
Mayer's test	To the pulp, add few drops of Mayer's reagent	A dull white colored precipitate indicates the presence of alkaloids
Hager's test	To the pulp, add few drops	Pale yellow colored precipitate indicates

	of Hager's reagent	presence of alkaloids.
Test for Carbohydrates		
Molish test	To the pulp, 1ml of ∝-napthol solution was added and Conc. Sulphuric acid was added to the sides of the test tube	Purple or reddish violet colour at the junction between the two liquids indicates the presence of carbohydrates
Fehling test	To the pulp, equal quantities of Fehling A&B were added .Upon heat gently	A brick red precipitate indicates the presence of carbohydrates.
Benedict's test	To 5ml of Benedict reagent, 8 drops of solution under test was added and mixed well. Then it was boiled vigorously for 2 minutes and cooled.	Red precipitate indicates the presence of carbohydrates
Test for Proteins		
Biuret test	To the pulp, 1ml of 40%NaOH and 2drops of 1% copper-sulphate solution was introduced	A violet color indicates the presence of proteins.
Xanthophoretic test	To the pulp, 1ml of conc. Nitric acid was added. When a white precipitate was formed, it is boiled and cooled. Then 20% of NaOH or ammonia was added.	Orange color indicates the presence of aromatic acids.
Lead acetate test	To the pulp, 1ml of lead acetate solution was added.	A white precipitate indicates the presence of proteins

Test for Amino acids		
Ninhydrin test	2 drops of freshly prepared 0.2% Ninhydrin reagent was added to the pulp and heated.	Development of blue color shows that may be the presence of proteins, peptides or amino acids.
Test for Steroids		
Liebermann burchard test	The pulp was dissolved in 2ml chloroform in dry test tube. 10 drops of acetic anhydride and 2 drops of conc. Sulphuric acid were added.	The solution becomes red and then blue and finally bluish green in color indicates the presence of steroids.
Test for Cardiac glycosides		
Keller-killiani test	Test sample was dissolved in acetic acid containing traces of ferric chloride and transferred to the surface of conc. Sulphuric acid.	At the junction, reddish brown colour was formed, which gradually becomes blue indicates the presence of cardiac glycosides.
Test for Saponins		
Foam test	About 1ml of formulation diluted separately with distilled water to 20ml and shaken in a graduated cylinder for half an hour.	A thick layer of foam indicates the presence of saponins.
Test for Phenolic compounds and Tannins		
Gelatin test	To 1mL of pulp add 1% gelatin solution containing 10% NaCl	Formation of white precipitate indicates presence of tannins.
Ferric chloride test	To the 1mL of pulp add add few drops of ferric chloride.	Formation of blue colour indicates presence of phenolics.

B.3. ESTIMATION OF TOTAL PHENOLIC CONTENT (SINGLETON et al., 1999)**)**

PROCEDURE

The total phenolic content of the natural fruits pulp formulation "FRUITITOP" was determined by using Folin-Ciocalteu reagent following a slightly modified method of SINGLETON et al., 1999). Methanolic solution of the extract in the concentration of 1 mg/ml was used in the analysis. Reaction mixture was prepared by pipetting out 0.5 ml volumes of the fruits pulp formulation (1mg/mL methanol) was mixed with 2 mL of the 10% Folin-Ciocalteu reagent then incubate at dark for 6mins and neutralized the reaction mixture with 4 mL of 7.5% sodium carbonate (Na_2CO_3) solution. Plotting calibration curve Gallic acid (20-100 µg/ml) was used as a reference standard. The reaction mixture was incubated at room temperature (RT) for 30 minutes with intermittent shaking for developing blue color. The absorbance of the resulting blue color was measured at 765 nm. The total phenolic contents were calculated from the linear equation of a standard curve prepared with gallic acid of various concentrations. The content of total phenolic compounds expressed as mg/g gallic acid equivalent (GAE) of fruit extract.

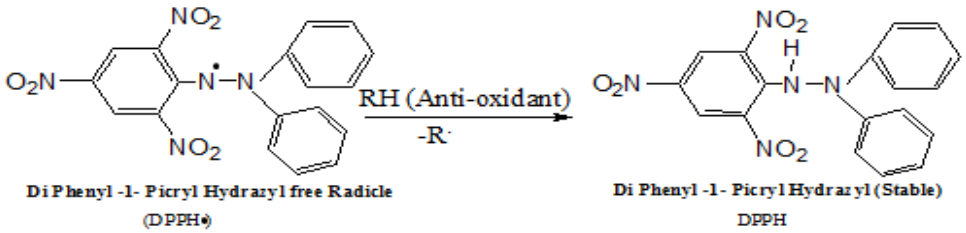
B4. SCREENING OF IN-VITRO PROTECTIVE EFFECT OF FRUITITOP

B4.1.ANTI-OXIDANT ACTIVITY ASSAY

B4.1.a. DPPH RADIC AL REDUCING ACTIVITY (Aquino et al 2010)

PRINCIPLE

This method is simple and sensitive method. Assay is based on the theory that hydrogen donor is an antioxidant. It measures compound that radical scavengers. Given below the reaction mechanism by which DPPH radicles accept hydrogen from antioxidant.



The DPPH is one of the few stable and commercially available organic nitrogen radicles. The antioxidant effect is proportional to the disappearance of DPPH radicle in the sample. The monitoring DPPH radicle with UV-Visible spectrometry has become the most commonly used method because of accuracy and simplicity. Addition of DPPH radicle to the test sample cause color turns from purple to yellow followed by the formation of DPPH upon absorption of hydrogen from the antioxidant. The antioxidant activity can be easily evaluated by following the decreasing UV absorption 517 nm.

REAGENTS:

- DPPH - Accurately weighed 3mg of DPPH· is dissolved in 25ml methanol (Stored in dark bottle protected from light)
- Methanol

PROCEDURE:

Different concentration of aqueous fraction (10, 20, 30, 40, 50, 60, 70, 80µg/ml) was added into the test tube. Add 187µl of freshly prepared DPPH free radicle solution. The volume was made up to 1ml with methanol. After that, test tubes are incubated in dark place at room temperature for 20 minutes. After the incubation the absorbance was read out in 517 nm. Methanol was used as a blank, Vitamin C was used as positive control and sample without drug was kept as control. The concentration of test materials to scavenge 50% DPPH radical (IC_{50} value) was calculated from the graph plotted with % inhibition against Concentration.

CALCULATION:

$$\textbf{\% inhibition} = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control} \times 100$$

B4.1.b. SUPEROXIDE RADICAL GENERATION BY RIBOFLAVIN PHOTO-REDUCTION: (Mc Cord and Fridovich (1969))

PRINCIPLE:

The superoxide anion radical scavenging activity was determined by nitro blue tetrazolium (NBT) reduction method of Mc Cord and Fridovich (1969), which depends on light induced super oxide radical generation by riboflavin. The assay is based on the ability of drug to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduction of riboflavin within the system. The superoxide radical thus generated reduce the NBT to a blue colored formazan complex..

REAGENTS:

- Sample drug
- Nitro blue tetrazolium (NBT) - 1.5mM (12.3mg/10ml)
- Riboflavin (freshly prepared) - 0.12mM (4.5mg/100ml)
- NaCN/EDTA - 0.0015% NaCN in 0.1M EDTA
- Phosphate buffer - 0.06M (pH 7.8)

PROCEDURE:

The 200µL of reaction mixture contained EDTA (0.1 M), 0.3mM NaCN, 50 µL of Riboflavin (0.12mM), 100µL NBT (1.5mM), and various concentrations of the natural fruits formulation in a final volume of 3ml was make up with Phosphate buffer (0.06 M, pH 7.8). The tubes were illuminated under incandescent lamp for 15min. The optical density at 560 nm was

measured before and after illumination. The inhibition of superoxide radical generation was determined by comparing the absorbance values of the control with that of natural fruits pulp formulation. Vitamin C was used as positive control. The concentration of natural fruits pulp formulation required to scavenge 50% superoxide anion (IC_{50} value) was then calculated.

CALCULATION:

$$\textbf{\% inhibition} = \frac{OD\ of\ control - OD\ of\ sample}{OD\ of\ control} \times 100$$

B.4.2.CYTOTOXICITY ASSAY

B.4.2.a. SHORT TERM CYTOTOXICITY (TRYPAN BLUE EXCLUSION METHOD) using Dalton's Ascites Lymphoma Cells.

PRINCIPLE

The drug at toxic concentration damages the cell and makes pores on membrane through which tryphan blue enters. The damaged cells are stained blue by tryphan blue stain and can be distinguished from viable cell.

MATERIALS REQUIRED

1. DLA (Dalton's Lymphoma Ascites) bearing mice
2. Phosphate buffer saline (PBS) pH 7.4
   - Na_2HPO_4 - 0.72gm
   - NaCl - 4gm
   - KCl - 0.1gm
   - Distilled water - 500ml
3. Tryphan blue (Prepared by mixing 100mg of tryphan blue and 900mg of NaCl in 100ml distilled water agitated overnight and filter and use it)
4. Microscope
5. Hemocytometer

PROCEDURE

Short term cytotoxicity was determined by Trypan blue dye exclusion method (Moldeus et al., 1978). DLA cells were aspirated from the peritoneal cavity of tumor bearing mice. The cells were washed three times using PBS below 1000rpm for 1min cell button using Pasteur pipette. The viability of cells was checked out using tryphan blue (all viability should be below 98%). Different dilution of 10^{-1}, 10^{-2} and 10^{-3} were made. The number of cells in the 10^{-2} dilution was counted using a hemocytometre and the cell number was adjusted to$1*10^6$ cells/ml. The experiment was setup by incubating different concentration of the drugs with $1*10^6$ cells. The final concentration of the assay mixture was made up to 1ml using PBS and was incubated at 37^0C for about 3hrs. 0.1ml of tryphan blue was added after incubation and further incubated for 3 min. The cell suspension (10 µL) was loaded on to a haemocytometer and observed under microscope. Live cells (non-stained cells) and dead cells (blue stained cells) were separately counted and percentage cell death was determined.

LONG TERM CYTOTOXICITY STUDIES

B. 4.2.b MTT **assay (**Mosmann 1983**)**

Maintaining of Cell lines and Biosafety analysis

MDAMB- 231 cells were obtained from National Centre for Cell Sciences, Pune, India. They were maintained in DMEM media, supplemented with 10% FBS at 37 ^oC and 5% CO_2.

Cytotoxicity assay protocol

MDA-MB- 231 cells ($1X10^5$) were seeded on 12 well plates. At subconfluency, cells were exposed to various concentrations of aqueous extract of Nutritop (10–100 µg/mL) over 24 hr periods to test their toxicity. The cell viability was assessed using 3-(4, 5- dimethyl thiazol-2-yl)- 2, 5-diphenyl tetrazolium bromide (Mosmann 1983). For this following incubation with additives MDA MB- 231cells were washed with PBS and MTT was added and incubated further for 4h. After the incubation, 500µL of solubilization reagent (5mL Triton-X 100, 45mL isopropanol and 1 drop HCl (12M)) was added and mixed well. Absorbance was taken at

570nm. The optical density of untreated control well was considered as 100% survival and percentage death of cells were calculated from the variation of OD in the treated wells.

B.5. **TOXICITY STUDIES**

B.5.a. **ACUTE TOXICITY STUD**

Experimental Protocol

- Guideline : OECD-420 fixed dose method
- CPCSEA Ref. No :ACRC/IAEC/2016/06-03
- Test : Limit test
- Species : Mus muscularis
- Strain : Swiss Albino mice
- Number of animals : 24 animals (6 for each group)
- Sex : Female
- Route of administration : Oral
- Duration : 3 hr close observation, followed by 14 days observation.
- Others : Body weight, mortality status, Water and feed consumption.
- Parameters : CNS, ANS and Behavioral changes.
- Blood collection : Not required. .
- Sacrifice : Sacrificed in 14^{th} day.

Table 8- Experimental Design of Acute Toxicity Study

GROUP	Number of Animals	DOSE (mg/kg)
Group 1	6	50
Group 2	6	300
Group 3	6	2000
Group 4	6	5000

STUDY DESIGN

Selection of Test animal

Female adult Swiss albino mice of 8-12 weeks are selected for the study. Non-pregnant and nulliparous animals were purchased from the Small Animal Breeding Station, Kerala Veterinary and Animal Science University, Mannuthy, Kerala, India and they are acclimatized for holding 1 week prior to dosing.

Housing and feeding conditions

Temperature - As per OECD GUIDELINE-420 the temperature of animal house is maintained at $22^0C \pm 3^0C$.

Humidity - The relative humidity of animal room maintained at 50-60% preferably not exceeds 70% (OECD guidelines-420, 2001). Otherwise there may be chances of developing lesions such as ring tail and food consumption may be increased.

Light – The sequence of light used is 12hrs light and 12hrs dark.

Caging – Polypropylene cages with solid bottom and walls. The lids are made up of stainless steel grill which is capable to hold both feed and water.

Feeding condition and feed – Sterile laboratory feed (ad libitum) and water daily. The feed used is brown colored chow diet.

Drug administration -Animals are fasted prior to dosing (food but not water should be withheld for overnight). After that animals are weighed and the test substance administered. The healthy Swiss albino mice has been taken and divided into 3 different groups. The test substance is administered in a single dose by oral gavages, using a curved and ball tipped stainless steel feeding needle.

Clinical observation

All animals were monitored continuously with special attention for 4hrs after dosing for signs of toxicity. Additional observations such as body weight changes, mortality, water consumption and feed intake are calculated are also done for the next 14 days for any other behavioral or clinical signs of toxicity.. At the end of the study, on the 15th day, all mice were sacrificed for necropsy examination.

Body weight

The body weights were recorded during the study and at scheduled up to the necropsy.

Feed and water consumption

Food consumption was assessed on a daily basis by weighing the feeds and it expressed as grams per mice per day. Water consumption was measured using 500ml measuring cylinder

PHARMACOLOGICAL SCREENING

The pharmacological screening of protective effects of FRUITITOP fresh fruits formulation was done by two methods such as in-vivo and in-vitro method.

SCREENING OF IN-VIVO PROTECTIVE EFFECTS OF FRUITITOP

The Female Swiss albino mice of average weight 25–30 gm used for this present work. The animals Swiss albino mice used for the experiment were maintained under standard laboratory conditions in an animal house of Amala cancer research centre (ACRC) approved by

the committee for the purpose of control and supervision on experiments on animals (Ref.No: ACRC/IAEC/2016/06-03) under 12hr dark/light cycle and controlled temperature 24±2^{0}C. They had free access to standard food and water ad libitum. The animals were acclimatized to the laboratory for a period of one week, before the commencement of experiment.

3B.6. DOSE CALCULATION

Contents	Solid part (%)	Moisture content (%)	Total part taken (gm)
Malus domestica	22.00	78.00	100
Vitis vinifera	26.3	73.70	100
Citrus sinensis	15.40	84.60	100
Punica granatum	12.40	87.60	100
Manilkara zapota	17.80	82.20	100
Total	93.9	406.1	500

Table No-8-Moisture content of fruits

The formulation consists of combination of five fruits of 100gm each. 500gm of fruits gives 450ml of pulp consists of 93.9gm of solid part (Table no.8). As fruits are so expensive, if an average 60kg body weight person is consuming 75ml of fruits pulp on daily basis gives 15.65gm solid particle consumption. Then human dose corresponding to 1kg body weight is 261 mg/kg. So, the animal dose equivalent to human dose will be 3217mg/kg, calculated by following formula **(Chang-Gue Son et al., 2010)**

CALCULATION:

Animal dose (mg/kg) = $Human\ eqivalent\ dose\left(\frac{mg}{kg}\right) * \frac{Human\ Km}{Animal\ Km}$

Human K_m = 37 Animal $K_{m\ (mice)}$ = 3

B.6. ANIMAL GROUPING

The animals (with body weights ranging from 25 to 30 gm.) were divided into 6 groups of 6 animals each. The study was divided in to two stages

STAGE 1

❖ **One month study without inducing stress:** In these two groups of animals was used each consists of 6 animals.

- **Group I: FRUITITOP** –Treated (FT)
- **Group II:** Normal – Untreated. (N1)

The Group **I** was treated with normal saline kept as normal and Group **II** were orally treated with the FRUITITOP at human dose equivalent to animal dose based on the body weight **(Chang-Gue Son et al 2010)** for a continuous time period of 30 days, The changes in body weight, feed and water consumption Hb and WBC count was recorded at every weeks. At the end of the treatment period, animals were fasted overnight and anesthetized by using chloroform and sacrificed. Blood, Brain, Heart, Kidney and Liver tissue were collected for various estimations.

STAGE 2

❖ **Two month study by inducing stress (chemotherapeutic drug- CTX):** In these four groups of animals was used each consists of 6 animals.

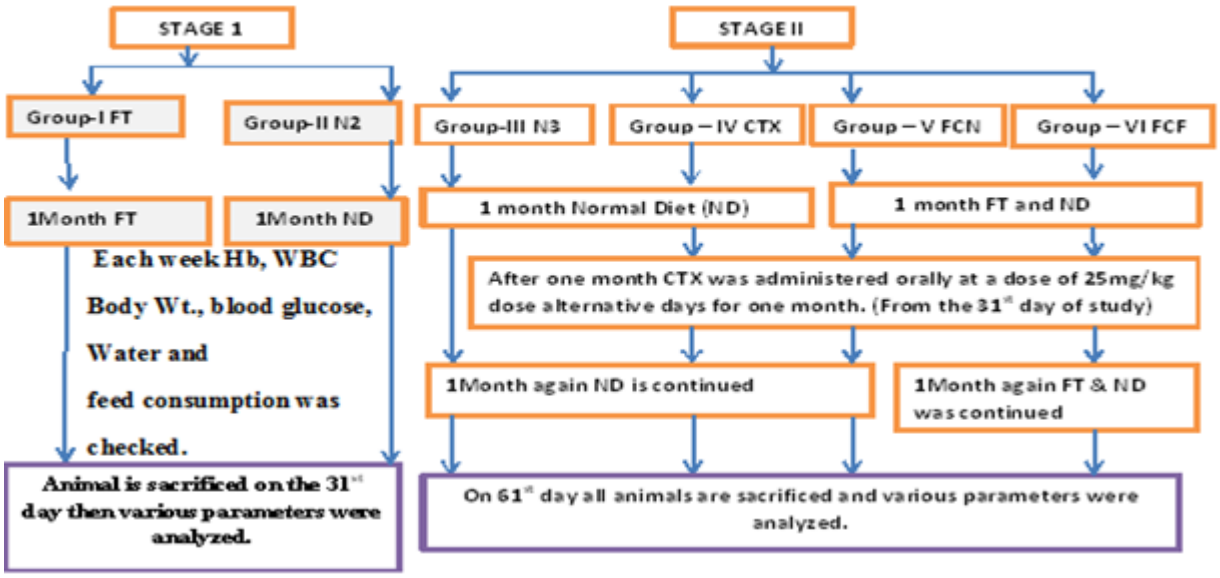
- **Group III:** Normal- Untreated.
- **Group IV:** Control- CTX treated (25 mg/kg body wt.) for one month (oral) for alternative days.
- **Group V:** This group was pretreated with **FRUITITOP** for one month followed by continuing **FRUITITOP** administration again for one month by inducing stress by chemotherapeutic drug CTX.

➢ **Group VI:** This group was pretreated with **FRUITITOP** for one month followed by continuing NORMAL DIET (ND) administration for one month by inducing stress by chemotherapeutic drug CTX.

The **Group III** was treated with normal saline kept as normal and **Group IV** was control group where the standard chemotherapeutic drug cyclophosphamide (**CTX)** was given orally at a dose of 25 mg/kg body weight alternative days for a period on one month.

Group V & VI: These groups was pretreated with **FRUITITOP** at dose of human dose equivalent to animal dose for a continuous time period of one month followed by continuing **FRUITITOP** consumption and **NORMAL FEED** consumption again for a period of one month in **Group V & VI**, by inducing stress by chemotherapeutic agent (**CTX)** at a dose of 25 mg/kg body weight administered via oral route in alternative days from the starting of second month of study. The changes in body weight, feed and water consumption Hemoglobin and WBC count was recorded at every weeks. At the end of the treatment period, animals were fasted overnight and anesthetized by using chloroform and sacrificed. Blood, Brain, Heart, Kidney and Liver tissue were collected for various estimations.

Fig3 -Schemtic representation of stage 1 and stage 2 studies



B.7. ESTIMATION OF BODY WEIGHT

The body weights of all the animals were measured, starting from the first day, and repeated every week, until sacrifice. On the 31st (Stage1) and 61st (Stage 2) day of the study, the animals were sacrificed and the blood and tissue parameters, relative organ weights as well as the bone marrow cellularity were checked.

B.8. DETERMINATION OF RELATIVE ORGAN WEIGHTS

After sacrifice, the liver, kidney, heart and brain were excised and weighed. The percentage weight of each organ, relative to the total body weight was determined.

B.9. DETERMINATION OF BONE MARROW CELLULARITY

Bone marrow cells from both femurs were collected in to phosphate buffer saline (PBS) containing goat serum. Both the femurs were dissected and bone marrow from each femur was flushed in to a solution containing 5ml PBS and 100µl goat serum then centrifuged at 1000-1500 rpm using cooling centrifuge (4^0C) for 10minutes supernatant is removed and sediment cell is taken. To the sediment cells 1ml PBS is added. From this100µl cells were taken out 900µl PBS was added. It should mixed well by proper shaking. The number of bone marrow cells was determined using a haemocytometer and expressed as total live cells ($\times 10^6$) /femur.

B.10. BLOOD PARAMETERS

B.10.a. GLUCOSE ESTIMATION

Blood sample was obtained through puncture tail vein and glucose was estimated on 0th day and day of sacrifice by using Accu-Check Glucometer.

B.10.b. DETERMINATION OF TOTAL WBC COUNT AND HAEMOGLOBIN LEVEL

During the course of the study, the hematological parameters of the animals were tracked on every week. For this, blood was collected from the caudal vein into heparinized tubes and total WBC count and hemoglobin level were checked.

B.10.c.DETERMINATION OF TOTAL COUNT

Principle:

The whole blood was diluted using a diluent which hemolysis red cells, leaving all the nucleated cells intact. The number of white cells in a known volume and known dilution were counted using a counting chamber.

Procedure:

About 0.02 ml of blood was added to 0.38 ml of diluting fluid and mixed well. The diluted blood was charged into a NEUBAUER counting chamber. After 3-4 min, the total number of white blood cells in the four large corner square chambers was counted.

CALCULATION:

Total WBC = (Number of cells counted x 50) / mm^3

B.10.d. DETERMINATION OF HAEMOGLOBIN (Hb) CONTENT

Cyanmethemoglobin method (Kit manufactured by AGAPPE Diagnostics).

Principle:

Hemoglobin (Hb) was treated with a reagent containing potassium ferric cyanide, potassium cyanide and potassium di hydrogen phosphate. The ferric cyanide forms methaemoglobin, which is converted to cyano-methaemoglobin by cyanide. The intensity of color formed is measured at 546 nm against blank. The optical density is directly proportional to the amount of hemoglobin present in blood.

Procedure:

Freshly collected whole blood 10µl of fresh whole blood was mixed with 2.5ml of the cyanmethemoglobin reagent. Blank is consisting only of cynomethemoglobin reagent. Shake

reagent solution slightly after adding the blood in order to avoid clumping of erythrocyte. Incubate for 45mins at room temperature measure the absorbance of specimen against reagent blank. The color will stable for 60mins do not expose to strong light. The optical density was measured at 546 nm against blank after 5 min incubation at room temperature. The OD of standard solution corresponding to 60 mg/dl hemoglobin at 546 nm was also read against reagent blank.

CALCULATION:

$$\textbf{Hemoglobin (gm/dL)} = \frac{\text{OD of treated x 60 x 0.251}}{OD\ of\ standard}$$

B.11. SERUM PARAMETERS

B.11.a. Blood sampling and serum separation:

Un- haemolysed blood sample was collected from the clean tail tips/ orbital sinus in eppendroff tubes from the anaesthetized animals. The blood was allowed to clot at room temperature 37ºC and centrifuge at 3000 rpm for 10 min to separate the serum and subjected to biochemical estimation.

B.11.b. Estimation of Aspartate aminotransferase (AST)/ SGOT

(Reitman and Frankel Method)

Aspartate aminotransferase (AST) is an end point colorimetric method for the estimation of enzyme activity. Kit manufactured by span Ltd. (Cogent) diagnostics Chennai, India).was used for this estimation.

Principle:

Aspartate aminotransferase, also known as Glutamate Oxaloacetate Transaminase (GOT) catalyses the transamination of L-aspartate and α keto glutarate to form oxaloacetate and

L- glutamate. Oxaloacetate formed is coupled with 2,4- Dinitrophenyl hydrazine to form hydrazone, a brown coloured complex in alkaline medium which can be colorimetrically measured.

α-KG + L-Aspartate ↔ oxaloacetate + L-glutamate

Oxaloacetate+ 2,4- DNPH ↔ corresponding hydrazone.

Reagents:

R1	Buffered aspartate –α KG substrate, pH-7.4	Phosphate buffer, aspartate ,α- KG
R2	2,4-DNPH colour reagent	2,4-dinitrophenyl hydrazine
R3	Sodium Hydroxide	Dilute 1 ml of R3(4N) to 10 mL with distilled water
R4	Working Standard	Sodium pyruvate 6mM(114U/L)

Procedure:

Reagent1 (0.25 ml) was taken and 0.05 ml of serum was added into the tube. Mixed well and incubated for about 60 minutes at **37**oC. 0.25 ml of R2 was added and mixed. It was then allowed to stand at room temperature for about 20 minutes. 2.5 ml of solution 1 was added into it and the absorbance was read at 505nm within 15 minutes by using the blank. Blank was prepared by the same procedure by using deionized water instead of serum. Standard and control was also processed.

CALCULATION

The enzyme activity was calculated as:-

$$\textbf{AST (GOT) activity in IU/L)} = \frac{[(\textbf{Abs of test} - \textbf{Abs of control})}{(\textbf{Abs of standard} - \textbf{Abs of blank})} \times \textbf{Conc. of the std.}$$

B.11.c. ESTIMATION OF CREATININE:-

Jaffe's Kinetic method

Kit manufactured by Asritha Diatech India (Euro) was used for this estimation.

Principle:

Creatinine is the catabolic product of creatinine phosphate which is used by the skeletal muscle. The daily production depends upon the muscular mass and it is excreted out of the body through kidney. Creatinine present in the sample generates a coloured complex upon reacting with sodium picrate.

creatinine + sodium picrate → creatinine-picrate complex

REAGENTS

- R1- Picric acid reagent
- R2- Alkaline buffer
- Creatinine Standard - 2 mg/ dl.

PROCEDURE

Working reagent (WR) was prepared by mixing equal volumes of R1 and R2. The reaction systems consisted of standard and test solutions. 1 ml of WR was dispensed to all the tubes, followed by the addition of 0.05 ml of creatinine standard and 0.05 ml of serum into the standard and test sample tubes respectively. Mixed well and read the absorbance of standard and test against distilled water at 520 nm after 30 sec (A_0) and 90 sec (A_1).

CALCULATION

The enzyme activity was calculated as:-

$$\textbf{Serum Creatinine (mg/dL)} = \frac{(\Delta AT)}{(\Delta As)} \times 2$$

$\Delta A_s = A_{S1} - A_{S0}$

$\Delta A_T = A_{TI} - A_{TO}$

ΔA for standard (S) and Test (T) was determined as:-

B.12. LIPID PROFILE

B.12.a. ESTIMATION OF CHOLESTEROL (Naito HK et al., 1984)

PRINCIPLE

The cholesterol estimation was done by using commercially available euro kit. Cholesterol esterase present in the working reagent will hydrolyze cholesterol to cholesterol esters in the serum. The cholesterol oxidase in the reagent cause formation free cholesterol by oxidation to form 4en-3-one with simultaneous production of hydrogen peroxide which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase (POD) to form red chormophore.

REAGENTS

R	PIPES PH 6.9 Phenol Cholesterol esterase (CHE) Cholesterol oxidase (CHOD) Peroxidase (POD) 4-Aminophenazone (4AP)	90 mmol/L 26 mmol/L 1000 U/L 300U/L 650 U/L 0.4 mmol/L
Cholesterol Standard	Cholesterol aqueous primary standard	

PROCEDURE

Working reagent (1000 µl) was pipetted out into a clean test tube. 10µl of sample was added into it. Mixed and incubated for 10 minutes at 37^{o}C. Standard was prepared by mixing 1000µl of working reagent and 10µl of standard. 1000µL of working reagent alone served as blank. After incubation absorbance was measured at 510nm (505-550nm).

CALCULATION

$$\textbf{Cholesterol (mg/dL in sample)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ standard} \times \textbf{concentration of Std}$$

(Concentration of standard = 200 mg/dL)

B.12.b. Estimation of triglycerides (Bucclo et al., 1973)

PRINCIPLE

Triglycerides incubated with lipoprotein lipase (LPL), liberate glycerol and free fatty acids. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine39 5-diphosphate (ADP) by glycerol kinase and ATP. Glycerol-3-phosphate (G3P) is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H2O2). Hydrogen peroxide (H2O2) reacts with 4- aminophenazone (4-AP) and p-chlorophenol inthe presence of peroxidase (POD) to give a red colored dye quinone. The intensity of the color formed is proportional to the triglycerides concentration in the sample.

REAGENTS

R	GOOD pH 6.3 p-chlorophenol LIPOPROTEIN LIPASE (LPL) GLYCEROL KINASE Glycerol -3-Oxidase 4 – Aminophenazone(4-AP) ATP	50 mmol/L 2 mmol/L 150000 U/L 500 U/L 3500 U/L 0.1 mmol/L 0.1 mmol/L
Triglycerides Standard	Triglycerides aqueous primary standard (200 mg/dL)	

PROCEDURE

Working reagent (1000 µl) was pipetted out into a clean test tube. Lipid extract (10µl) was added into it. Mixed and incubated for 10 minutes at 37^{o}C. Standard was prepared by mixing 1000µl of working reagent and 10µl of standard. 1000µL of working reagent alone served as blank. After incubation absorbance was measured at 505nm (490-550nm).

CALCULATION

$$\textbf{Triglycerides (mg/dL in sample)} = \frac{Absorbance\ of\ test}{Absorbance\ of\ calibrator} \times 200$$

B.12.c. ESTIMATION OF HDL CHOLESTEROL (**Kaplan et al**., 1984)

Principle

Direct estimation of HDLc levels in serum followed the method that depends on the properties of detergent which solubilize only the HDL, so that the HDLc released to react with the cholesterol esterase, cholesterol oxidase and chromogens to give color. The non HDL lipoproteins LDL, VLDL and chylomicrons are inhibited from reacting with the enzymes due to the absorption of the detergent on their surfaces. The intensity of the purple color formed is proportional to the HDLc concentration in the sample.

REAGENTS

R1	GOOD (pH=7) Cholesterol oxidase <1000 U/L DSBmT <1Mm
R2	GOOD (pH=7) Cholesterol oxidase <1500 U/L 4- Aminoantipyrene <1mM Detregent <2 % Ascorbic Oxidase <3000 U/L Peroxidase <1300 U/L
HDLc cal	Calibrator: Lyophilised human serum. Dissolve the contents with 1 mL of distilled water

PROCEDURE

Reagent R1 (450 µl) was taken in a test tube and 10 µl of sample was added into it. Mixed and incubated for 5 minutes at **37**^{o}C. Then 150 µl of R2 was added, mixed and incubated for 5 min. at **37^{o}**C. Absorbance was read at 600-700nm against appropriate blank

CALCULATION

$$\textbf{HDL}_{\textbf{C}}\textbf{ mg/dL in sample} = \frac{Absorbance\ of\ test}{Absorbance\ of\ calibrator} \times \textbf{39.2 (Calibrator conc.)}$$

B.12.d. ESTIMATION OF LDL CHOLESTROL

LDL cholesterol (LDL_C**):** LDL_C was calculated by using the formula (Fried-Wald equation)

CALCULATION

LDL_C mg/dL in sample = Total cholesterol – [HDL cholesterol – Triglycerides/5].

LDL_C level in plasma was expressed as mg/dL.

B.13. ESTIMATION OF PROTEIN (LOWRY'S METHOD)

Principle:

Under alkaline conditions, the divalent copper ion reacts with peptide nitrogen and the get reduced to a monovalent ion. Monovalent copper ion oxidizes tyrosine, tryptophan, and cysteine that reduces Folin-ciocalteau phenol reagent to an unstable blue product with absorption maxima at 570 nm.

Protein + Cu^{++} → Blue- violet colored complex

The intensity of the color formed is directly proportional to the amount of total proteins present in the sample.

Reagents:

- A. 2% Na2CO3 in 0.1N NaOH- (2gm of Na2CO3 dissolved in 0.399gm of NaOH/100ml D.W)
- B.- 1% Sodium potassium tartrate (0.1% in 100)
- C.- 0.5% CuSO45H2O in H2O (50mg/10ml D.W)

- Solution X was prepared by mixing 48ml of A+1ml of B+1ml of C
- Solution Y prepared by dissolving 5ml of folin-reagent mixed with 5ml of D.W.

Procedure:

Tissue homogenate (10µl, 10%) was diluted to 990 µl with distilled water and mixed with 5 ml of solution X. The mixture was incubated at room temperature for 10 min. To this solution 0.5ml of solution Y was added, mixed well and kept at room temperature for 30 min. the absorbance was read at 660 nm. The amount of protein was calculated from standard curve of BSA.

B.14. TISSUE ACTIVITY

B.14.a. PREPARATION OF TISSUE HOMOGENATES (LIVER & KIDNEY)

On 31st (stage-1) and 61st (stage-2) day, animals were sacrificed respectively. Liver and kidney were excised and rinsed thoroughly in ice cold normal saline solution to remove the blood. They were gently blotted between the folds of thoroughly in ice-cold saline to remove any blood traces. They were then gently blotted between the folds of a filter paper and weighed in an analytical balance. 10% of homogenate was prepared in 0.05M phosphate buffer (pH 7) using a Polytron homogenizer at ice cold condition. The homogenate was centrifuged at 6,000 rpm for 15-20minutes in a cooling centrifuge for removing the cell debris, unbroken cells, nuclei, erythrocytes and mitochondria. The supernatant was used for the estimation of Superoxide dismutase (SOD), lipid peroxidation (LPO), Catalase, total protein, Glutathione peroxidase (GPx) and Glutathione (GSH).

TISSUE ANTI-OXIDANT PARAMETERS

B.14.b. Estimation of Tissue Superoxide Dismutase (SOD) Activity

Determined by the method of Mc cord, Bean Champ and Fridovich (1969)

Principle:

The assay is based on the ability of enzyme to inhibit the reduction of nitro blue tetrazolium (NBT) by Superoxide, which is generated by the reaction of photo reduced riboflavin with oxygen.

Reagents:

- SOD buffer - Phosphate buffer (67mM) pH 7.8
- EDTA/KCN - (3.7gm EDTA in 100ml D.W + 115µl KCN (1%)) (0.1M)
- Riboflavin - (2mM), stored cold condition in a dark bottle (4.52mg in 100ml D.W)
- NBT - (1.5mM) (12.3mg/10ml buffer) stored cold condition.

Procedure:

For each sample to be assayed, the amount of enzyme added to the medium was kept below 1 unit of enzyme activity. Incubation medium contained 20µl of tissue homogenate, 200µl EDTA/KCN, 100 µl NBT, and 50 µl riboflavin and phosphate buffer to give a total volume of 3ml. The tube without animal sample was kept as control. OD value is taken before adding riboflavin. Riboflavin was added after the tubes were brought to room temperature. Then the tubes were placed in a bright box where they received uniform illumination for 15mins. Blue color formed after illumination will be decreased in sample which contains SOD. The optical densities were measured at 560nm. One unit of enzyme activity is defined as amount of enzyme giving 50% inhibition of the reduction of NBT and expressed as units/mg. protein.

B.14.c. Estimation of Glutathione (GSH)

Determined by the method of Moron et al., 1979

Principle:

GSH is measured by its reaction with DTNB to give a yellow colored complex with maximum absorption at 412nm. TCA was added to precipitate protein.

Reagents:

- 25% TCA (25gm in 100ml D.W)
- 5% TCA (5gm in 100ml D.W)
- 0.2M GSH buffer (pH 8.0)
- 0.6 Mm DTNB (5, 5'-dithiobis 2- nitro benzoic acid) (4.8mg/20ml buffer)

Procedure:

To 100µl of tissue homogenate 50µl of 25% TCA was added to precipitate proteins. The test tubes were cooled by keeping on ice for 5min and the mixture was further diluted with 0.3ml of 5% TCA; centrifuged 2000 rpm for 10 minutes and 150µl of clear resultant supernatant was taken for GSH estimation. The volume of the aliquot was made up to 1 ml with 350µl of 0.2M phosphate buffer (pH 8.0) and 1ml of freshly prepared 0.6mM DTNB was added. Reaction mixture is incubated for 5mins at room temperature. The intensity of yellow color formed by DTNB addition was read at 412 nm with distilled water as reference. Standard curve of GSH was prepared using concentrations varying from 5-100 nmol in 5% TCA for each assay. Value was expressed as nmol / mg protein.

B.14.d. Estimation of Glutathione Peroxidase (GPx) Activity

Determined by Batemann et al., 1974

Principle:

Glutathione peroxidase degrades H_2O_2 in presence of glutathione (GSH) thereby depleting it. GSH remaining is measured using DTNB, which gives a colored complex. NaN_3 is added in the reaction mixture to inhibit the action of catalase enzyme otherwise lipid hydro peroxide radical will form in the system is scavenged by catalase rather than glutathione peroxidase H2PO3 is act as a precipitant of protein in the solution.

Reagents:

- 0.1M Phosphate buffer (pH 7.0)

- 0.2 mM GSH - 7.7mg/5ml of phosphate buffer
- Sodium azide - 25mM (8.125mg/5ml) should be freshly prepared
- H_2O_2 - 1.25mM (14.2µl 100ml Distilled water)
- Na_2HPO_4 - 0.4M (5.678gm/100ML Distilled water)
- DTNB (5, 5'-dithiobis (2-nitrobenzoic acid) - 1Mm (9.91mg/25ml buffer)
- Meta-phosphoric acid (HPO_3) - (1.67%) 417mg/25ml buffer

Procedure:

The reaction mixture containing 0.1 ml each of tissue sample, GSH, NaN_3 and H_2O_2 was made up to a total volume of 2.5 ml using phosphate buffer and was incubated at 37°C for 6 minutes. After addition of 2 ml. of 1.67 % HPO_3, this mixture was centrifuged at 800×g for 15 minutes. 667µl of the supernatant was added to a mixture of 1ml of Na_2HPO_4 and DTNB. After 10 minutes of incubation at 37°C, the absorbance of the reaction mixture was measured at 412 nm, with distilled water as reference. The enzyme activity was expressed as units/mg protein.

B.14.e. Estimation of TBARS

Determined by the method of Ohkawa et al., 1979

Principle:

Malondialdehyde (MDA) produced during peroxidation can react with thiobarbituric acid (TBA) reagent to form a pink colored product which has an absorption maximum at 532nm. The assay is calibrated with 1,1,3,3, tetra meth-oxy propane, which on hydrolysis produces malondialdehyde. The results are expressed in terms of the amount of malondialdehyde produced during the reaction.

Reagents:

- SDS - 8%
- TBA - 0.8% (should heat slightly to dissolve in water before adding)
- Acetic acid- 20% pH 3.5, (20ml glacial acetic acid and 80ml D.W)

Procedure:

About 200µl of the tissue homogenate (25%) in 100µl SDS (8%), 0.75ml (20%) acetic acid, 0.75ml (0.8%) thiobarbituric acid and final reaction volume was made up to 2ml by using distilled water. The reaction mixture was incubated at 100^0C for 1hr. Cool all the test tube add 0.5ml of distilled water. Rotex well and centrifuge at 2000rpm for 15 minutes. Supernatant is taken for reading OD value at 532nm. The amount of MDA formed was expressed as nmol/mg. protein.

B.14.f. ESTIMATION OF CATALASE ACTIVITY

Determined by Beers and Sizer method (1952)

Principle: The decomposition of H_2O_2 catalyzed by catalase in to H_2O_2 and O_2 .Decomposition of H_2O_2 can be followed directly by the decrease in absorbance of H2O2 at 240 nm. The difference in absorbance per unit time is measure of catalase activity. The decomposition of H_2O_2 catalyzed by catalase can be followed by spectroscopy due to the absorbance of H_2O_2 in the region at 240nm. The molar extinction coefficient for H_2O_2 is 43.6per minute. One unit decomposed one micromoles of H_2O_2 per minute at 25^0C and pH 7 under the specific conditions. H_2O_2 is formed in eukaryotic cell as a product of various oxidase and super-oxidase dismutase reaction.

REAGENTS

Diluted stock hydrogen peroxide solution 1mM H_2O_2 prepare in dark bottle

Catalase buffer- 0.05M Phosphate buffer pH-7

PROCEDURE

At 240 nm the absorbance of the diluted hydrogen peroxide sample was recorded against quartz cuvette containing deionized water or initial OD of phosphate buffer was noted. 100 µl of tissue homogenate was taken, to this 1.9ml phosphate buffer and 1ml hydrogen peroxide solution (should added at the time of reading) added respectively. OD value is taken at 240nm. The decrease in absorbance was measured for 3 minutes at 60 sec interval after the addition of 100 µl of tissue. The reference cuvette contains 2ml of buffer and 1ml of H_2O_2. CAT activity was calculated using the formula and expressed as K/mg protein

Where K = Rate constant of 1st order reaction

CALCULATION:

$$\text{Catalase} = \frac{\Delta \text{OD} / 3}{\text{mg protein in sample}} \times 1000$$

Where ΔOD = initial OD – final OD.

B.15. HISTOPATHOLOGY

A portion of kidney and liver belonging to one animal in each group was preserved in 10% formaldehyde solution for histopathological studies. Hematoxylin and eosin were used for staining; later, the histopathological slides were photographed.

B.17. STATISTICS

The experimental results are presented as Mean ± SD for 6 animals in each group. Statistical evaluation of the data was done by one way ANOVA followed by Dunnet's t-test. Results were considered statistically significant when $p<0.05$.

6. RESULTS

The results of study was represented as mean ± SD of 6 animals each and the one way Analysis of variance (ANOVA) was calculated using "Graph pad INSTAT" software.

1. CHARACTERISTICS OF "FRUITITOP"

The organoleptic character of FRUITITOP such as color, odor, and taste was carried out whose results are given in (Table: 10). There were no characteristic changes happen in the colour, odour, taste and pH during storage period.

Table No: 10 "CHARACTERISTICS OF "FRUITITOP"

Sl.No	Parameter	FRUITITOP
1	Color	Ochre yellow
2	Odor	Fruity an pleasant
3	Taste	Mild bitter astringent and sweetish
4	pH	4.2

2. PRELIMINARY PHYTOCHEMICAL STUDIES

Natural fruits combination FRUITITOP were subjected to various phytochemical tests, to determine the presence of active constituents. The tested sample are positive to alkaloids, flavonoids, phenolic compounds, tannins, carbohydrates, proteins and amino acids results are given in (Table: 11).

Table No: 11, PRELIMINARY PHYTOCHEMICAL STUDIES

SL.NO	TEST	FRUITITOP
1	Carbohydrates	+
2	Proteins	+

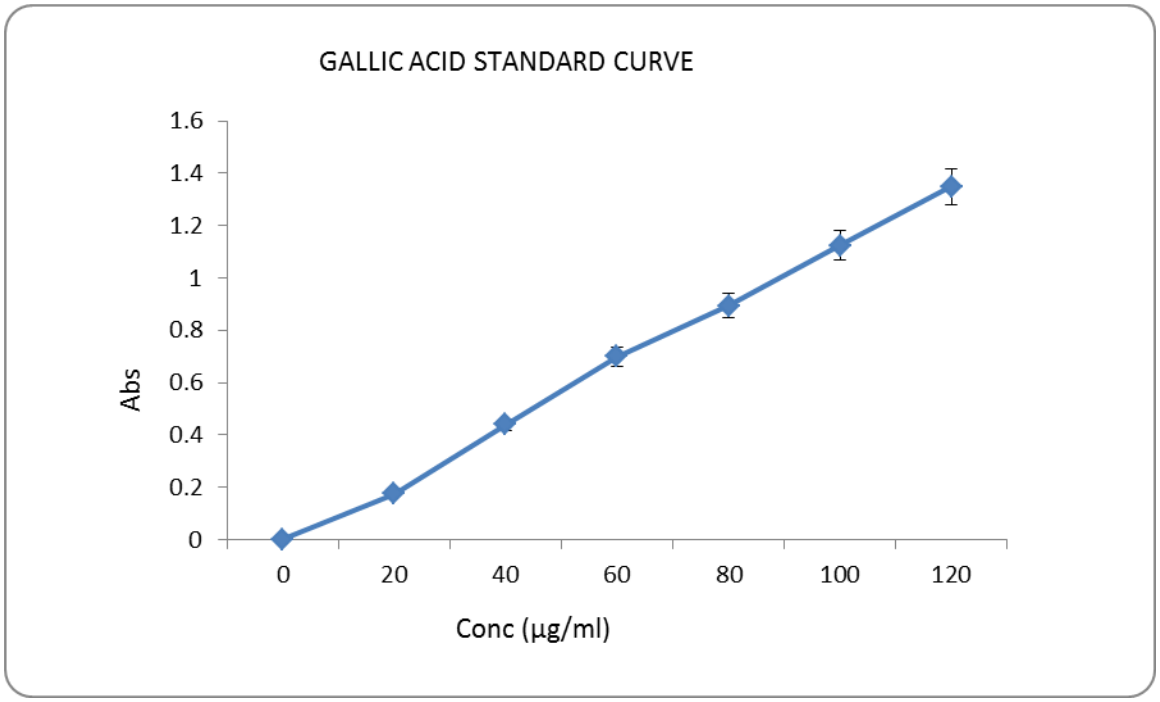
3	Amino acids	+
4	Alkaloids	+
5	Flavonoids	+
6	Cardiac glycoside	-
7	Steroids	-
8	Saponins	-
9	Phenolic compounds	+
10	Tannins	+

(+) indicates presence and (-) indicates absence compound.

ESTIMATION OF PHEONOLIC CONTENTS

In the estimation of absorbance of test compound (FRUTSITOP) was 0.412 for 10mg of sample. The total phenolic content of test compound was calculated from the standard curve plotted for Gallic acid, found significant phenolic content of 47.36±1.21µg GAE/mL (Fig: 4). The values are expressed as ± SD of three samples.

Fig: 4, ESTIMATION OF PHEONOLIC CONTENT



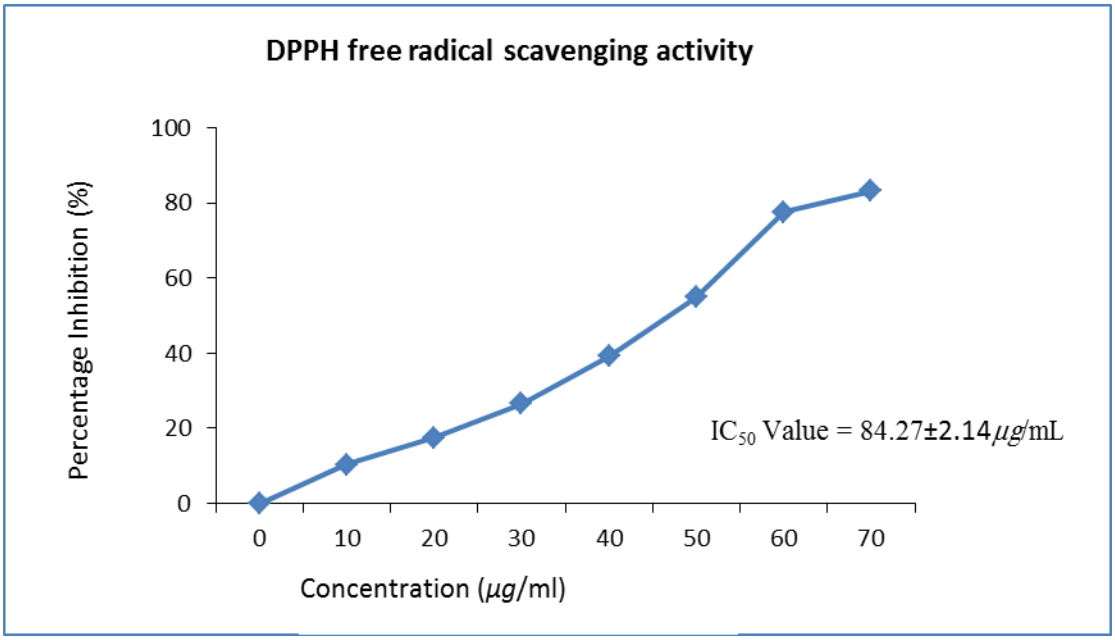
4. IN-VITRO EXPERIMENTS

4A. IN-VITRO ANTI-OXIDANT STUDIES

4A.1. DPPH RADICAL SCAVENGING ACTIVITY

The DPPH radical was effectively scavenged by various concentration of aqueous fraction of FRUTSITOP. A dose dependent reduction of was observed within the range of concentrations (0-100μg/ml) of reaction system. The IC_{50} value of FRUITITOP formulation was found to be. 84.27μg/ml. Vitamin C which was used as the positive control exhibited an IC_{50} value of 4.70 ±2.14 μg/ml (Figure.5). The aqueous fraction has no significant changes in IC_{50} values during long term storage, the values are expressed as ± SD of 3 reading.

Figure 5: DPPH RADICAL SCAVENGING ACTIVITY



4A.2. SUPEROXIDE RADICAL SCAVENGING ACTIVITY

Superoxide generated in the photo reduction of riboflavin was effectively inhibited by the addition of varying concentrations (5-50 µL/ml) of fruit juice. The concentration of the

FRUITITOP needed to scavenge 50% superoxide anion (IC_{50}) was found to be 92.62 ± 3.42 μg/ml (Figure 6) Vitamin C which was used as a positive control had an IC_{50} value of 65.31 ±2.54μg/ml.

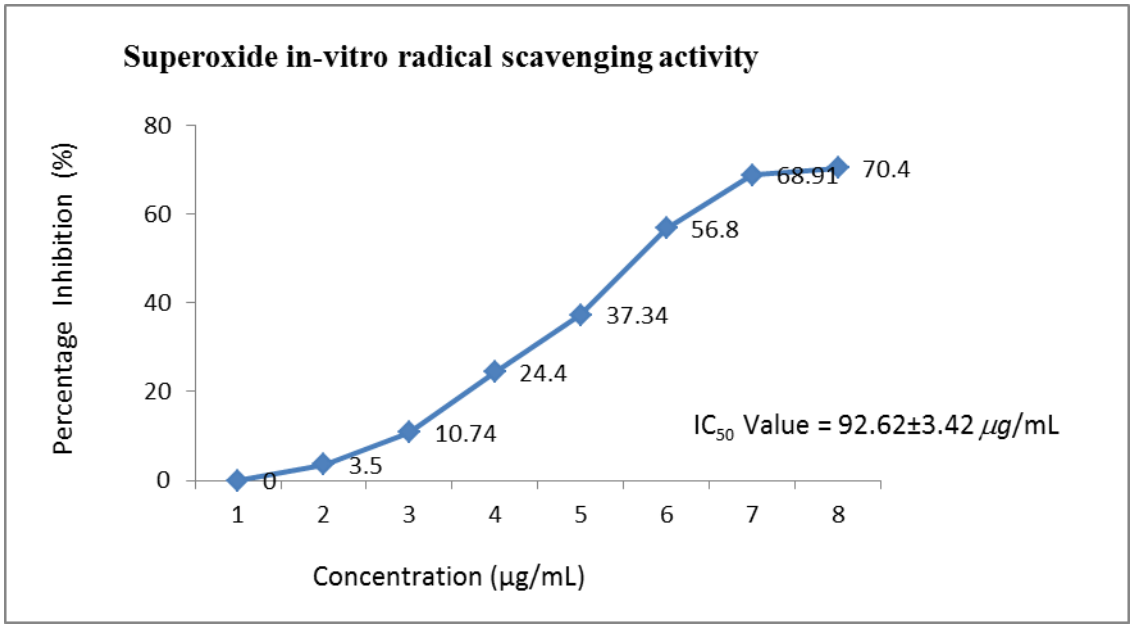


Figure 6: Effect of the FRUTSITOP on Superoxide *in vitro* Radical Scavenging. The reaction mixture contain various phytochemicals were illuminated for 15mins the absorbance was measured before and after illumination positive values are expressed ± SD of 3 reading.

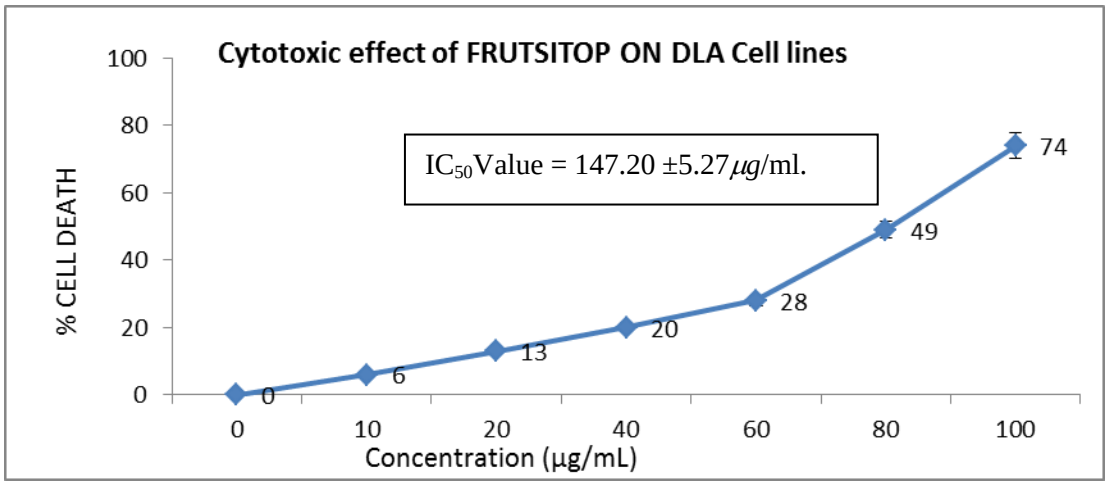
4B. IN-VITRO CYTOTOXICITY STUDIES

4B.1 Short term Cytotoxicity

ACTIVITY OF FRUITITOP AGAINST – DALTON'S ASCITIS LYMPHOMA

The short term cytotoxicity of FRUTSITOP was studied on DLA cell lines, a dose dependent cell death was observed within the range of concentrations (0-100μg/ml) of reaction system The IC_{50} value of FRUITITOP was found to be 147.20 ±5.27μg/ml.(Figure 7)

Fig.7- Short term Cytotoxicity effect of FRUITITOP against – DALTON'S ASCITIS LYMPHOMA

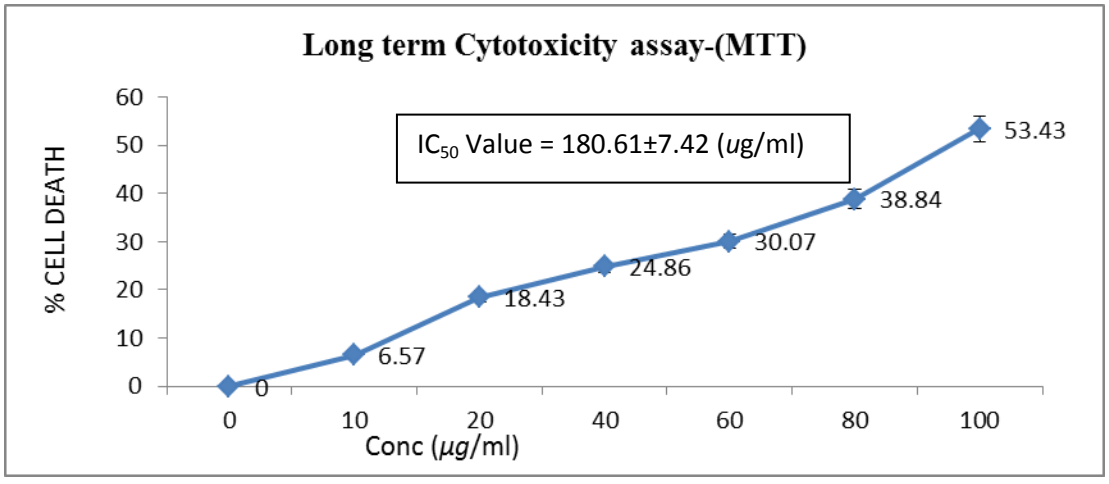


4B.2 Long term Cytotoxicity

ACTIVITY OF FRUITITOP AGAINST – MDA-MB 231(Triple negative human breast cancer) Cell lines.

The Long term cytotoxicity of FRUTSITOP was studied on **MDA-MB 231** cell lines, a cell death was observed within the range of concentrations (0-100μg/ml) of reaction system (Figure 8). The IC_{50} value of FRUITITOP was observed at the conc. of 180.61 ±7.42 (μg/ml).

Fig-8, Long term Cytotoxicity effect of FRUITITOP against – MDA-MB 231(triple negative human breast cancer) cell line



TOXICITY STUDIES

5. ACUTE TOXICITY

Acute toxicity experiment is conducted on female healthy mice according to OECD guidelines 423. During the acute toxicity studies the FRUITITOP was administered orally and the animals were observed for mortality, changes in ANS, CNS and various other behavioral responses. There was no mortality observed even at 2000mg/kg and 5000mg/kg of the FRUITITOP. All the animals were found to be normal during the experimental period. This observation revealed that the FRUITITOP did not show any symptoms of toxicity and mortality up to 2000mg/kg dose & 5000mg/kg dose and was found to be very safe up to 5000mg/kg of body weight known as maximum tolerated dose (MTD) as per OECD guidelines 420. Hence from this the animal dose corresponding to human dose is selected for the further pharmacological studies. Results of acute toxicity studies were given in (table: 11) and various behavioral response of acute toxicity study were tabulated in (Table: 13).

TOXICITY STUDIES

TABLE: 12, RESULTS OF ACUTE TOXICITY STUDIES

Drug	Group	Dose (Mg/Kg)	Mortality & symptoms of toxicity	Body Weight (gms)		
				Day 0	Day 7	Day 14
FRUISITOP	A	50	-	27.07±1.24	27.12±0.64	27.42±0.75
	B	300	-	27.17±1.31	26.88±1.16	27.60±1.23
	C	2000	-	26.91±1.03	26.90±1.06	27.08±0.71
	D	5000	-	26.37±1.23	26.52±1.04	26.40±0.87

Table No: 13, BEHAVIORAL RESPONSES OF ACUTE TOXICITY OF FRUTSITOP

SL.NO	Parameters	Observation
1	Urination/ Defecation	Normal
2	Light reflex	Normal
3	Lacrimation	Normal
4	Body position	Normal
5	Auditory response	Normal
6	Alertness	Normal
7	Tactile response	Normal
8	Eyelid closure	Normal
9	Gait	Normal
10	Locomotor activity	Normal

6. EFFECT OF FRUITITOP ON BODY WEIGHT, FOOD AND WATER CONSUMPTION:

Results revealed that, the oral FRUITITOP administration in groups IV, V & VI produce significant changes in the body weight of the animals during the study. This study shows that FRUTSITOP has the ability reduce the weight of the animals (Table 14). However, there were no significant changes in food and water consumption among rats of the experimental groups.

Table: 14 - Effect of FRUITITOP on body weight

STAGE I STUDIES				
	1st week	**2nd week**	**3rd week**	**4th week**
G-I FT	25.07±1.024	24.45±1.011	23.67±1.098	22.78±1.101
G-II N1	25.23±1.140	25.24±1.134	25.61±1.131	25.73±1.128
STAGE II STUDIES				

	2nd month-1st week	**2nd month -2nd week**	**2nd month -3rd week**	**2nd month -4th week**
G-III N3	26.63±2.67	26.48±2.53	26.92±2.05	26.90±2.10
G-IV CTX	26.68±1.29	24.11±1.39	22.84±1.48	21.08±1.52
G-V FCN	22.66±2.75	20.20±3.33	18.35±3.11	17.65±2.41
G-VI FCF	22.46±1.47	19.83±2.40	19.32±1.88	16.50±2.08

7. EFFECT OF FRUITITOP ON ORGAN WEIGHT:

The weight of heart, kidney, liver and brain of all animals of all group were found to be in similar range to that of the normal group. There are no significant changes in the organ weight compared to the control, treated and normal groups (Table 15).

Table: 15 - Effect of FRUITITOP on organ weight

	HEART	KIDNEY	LIVER	BRAIN
G-I FT	0.110±0.065	0.281±0.006	1.152±0.185	0.411±0.012
G-II N1	0.114±0.015	0.258±0.014	1.299±0.232	0.413±0.022
G-III N3	0.121±0.034	0.269±0.009	1.367±0.278	0.419±0.014
G-IV CTX	0.120±0.042	0.267±0.006	1.312±0.082	0.418±0.009
G-V FCN	0.118±0.064	0.253±0.003	1.237±0.071	0.415±0.002
G-VI FCF	0.119±0.094	0.257±0.001	1.354±0.001	0.413±0.002

8. EFFECT OF FRUITITOP ON TOTAL WBC COUNT:-

The results revealed that the control animals treated with cyclophosphamide alone showed lower levels of WBC count, when compared to the normal reference group. On treatment with FRUITITOP, the WBC levels of Group II, V & VI shows slight variation in WBC count (Table 16).

Table: 16 - Effect of FRUITITOP on Total WBC Count

STAGE l STUDIES				
	1st week	**2nd week**	**3rd week**	**4th week**
G-I FT	10800±0.558	11149±0.547	11220±0.687	11879±0.625
G-II N1	10467±.0.412	10122±0.552	11303±0.456	10789±0.356
STAGE II STUDIES				
	2nd month-1st week	**2nd month -2nd week**	**2nd month -3rd week**	**2nd month -4th week**
G-III N3	10587±0.471	11024±0.547	12035±0.425	12060±0.541
G-IV CTX	12120±0.321	9241±0.553	6450±0.354	5240±0.400
G-V FCN	11004±0.726	10120±0.208	9320±0.450	8910±0.208
G-VI FCF	11024±0.654	10985±0.325	9960±0.400	9620±0.313

9. EFFECT OF FRUITITOP ON HAEMOGLOBIN LEVEL:-

The group treated with Cyclophosphamide did not show any significant difference in the hemoglobin. The FRUITITOP treated groups also did not show any notable changes in the level of hemoglobin (Table 17).

Table: 17 - Effect of FRUITITOP on Hemoglobin level

STAGE l STUDIES				
	1st week	**2nd week**	**3rd week**	**4th week**
G-I FT	12.68±0.874	12.62±0.478	12.86±0.574	12.77±0.675
G-II N1	12.36±0.365	12.37±0.412	12.69±.201	12.89±0.447
STAGE II STUDIES				

	2nd month-1st week	**2nd month -2nd week**	**2nd month -3rd week**	**2nd month -4th week**
G-III N3	14.5±0.214	13.8±0.441	13.5±0.324	13.7±0.321
G-IV CTX	12.7±0.201	11.8±0.287	12.1±0.514	12.5±0.321
G-V FCN	12.2±0.685	11.4±0.547	11.9±0.521	12.9±0.411
G-VI FCF	13.1±0.440	12.6±0.345	12.5±0.301	12.9±0.236

10. EFFECT OF FRUITITOP ON SERUM PARAMETERS:

10a. ESTIMATION OF ASPARTATE AMINOTRANSFERASE (AST/ SGOT):-

The results revealed that the Cyclophosphamide treated control animals group alone showed higher levels of the SGOT, the level were $139.265\pm6.367_{lU/L}$ ($P<0.001$), compared to the normal reference group ($114.324\pm5.457_{lU/L}$). On treatment with FRUITITOP, there was a decrease in the level of SGOT with values recorded as $131.486\pm5.382_{IU/L}$ and $125.32\pm7.82_{IU/L}$ respectively for G-V and G-VI groups (Fig 9) compared to normal (G-III).

10b. ESTIMATION OF SERUM CREATININE:-

The level of creatinine was found to be 0.726 ± 0.048 mg/dL in the normal mice which shows a significant increase to about1.115± 0.081 mg/dL cyclophosphamide alone treated control group (G-III) animals ($P<0.001$). The FRUITITOP treatment groups V & VI showed significant reduction in the creatinine level, i.e; 1.003 ± 0.026 mg/dl and 0.974 ±0.031 mg/dL respectively (Fig 10) while compared to (G-III).

ESTIMATION OF ASPARTATE AMINOTRANSFERASE (AST/ SGOT):-

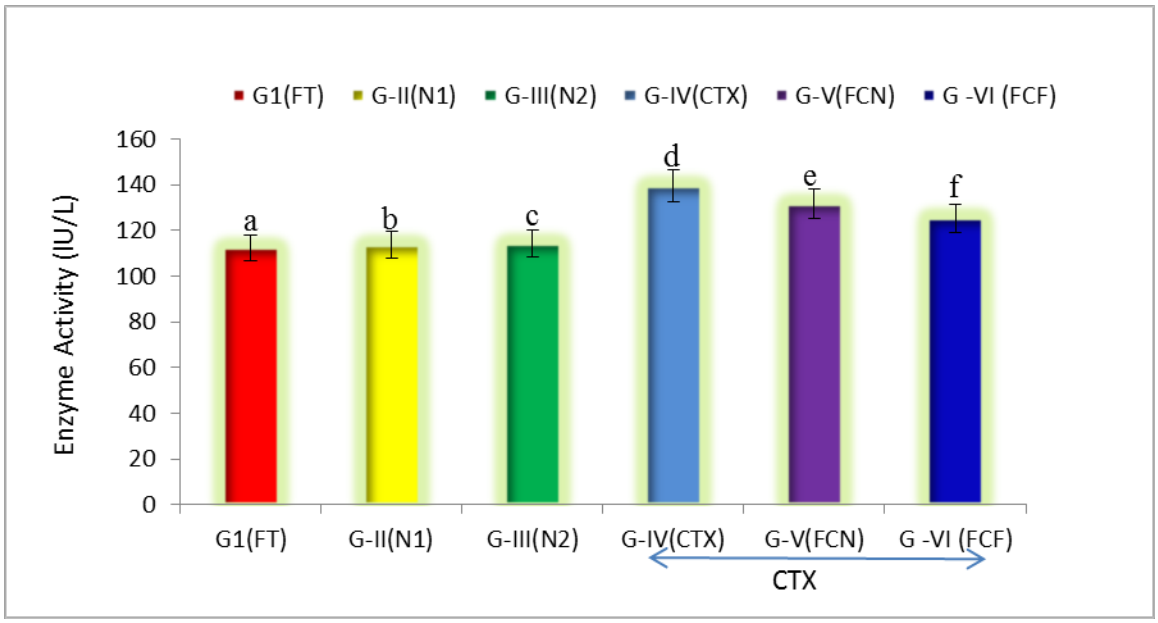


Fig: 9 - Effect of FRUITITOP on aspartate aminotransferase (AST/ SGOT), d = $p<0.001$, e = $p<0.001$ and f = $p<0.05$ compared to normal (c), and there is no significant difference between (a) compared to normal N_1 (b).

ESTIMATION OF SERUM CREATININE

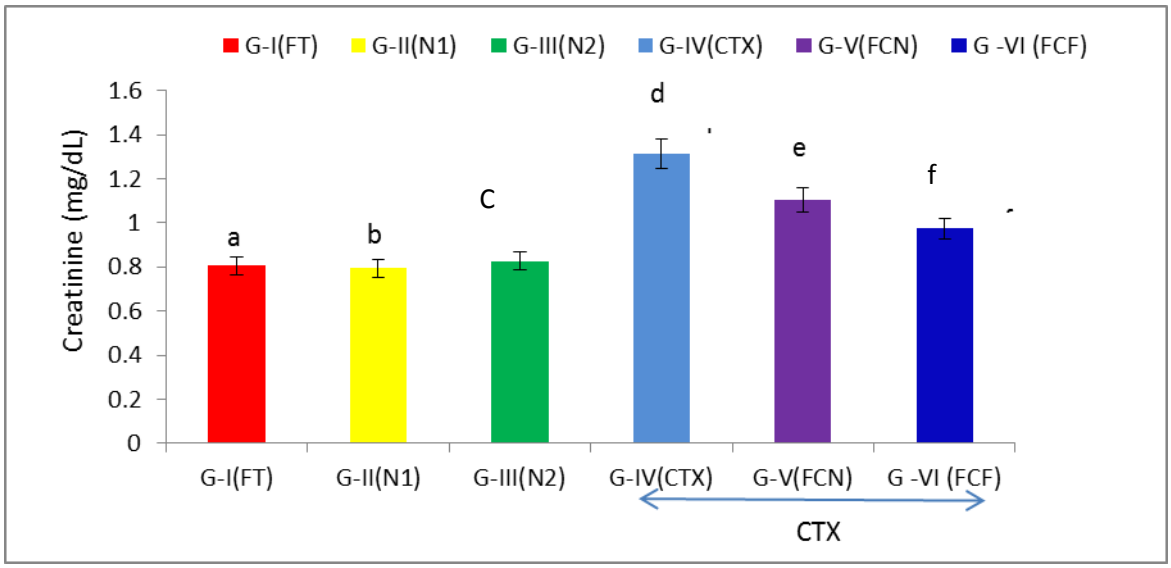


Fig: 10- Effect of FRUITITOP on Creatinine

d = $p<0.001$, e = $p<0.01$ and f = $p<0.01$ compared to normal (c). No significant difference between (a) and (b).

10c. SERUM LIPID PROFILE

Serum total **cholesterol** levels in stage-1 studies, there were slight significant changes noticed in the in the FRUITITOP treated group (**G-I)** 65.14±5.9 mg/dL and normal (**G-II)** group 73.10±4.5mg/dL But, in stage-II studies, total serum cholesterol level of (**G-III)** normal mice was 69.74±5.55mg/dL. In the animals fed cyclophosphamide (**G-IV)** alone the level was 78.10±6.33mg/dL. There were significant changes noticed in the serum total cholesterol levels in the treated groups. In one month FTUITSITOP fed animals (**G-V)**, the level was 53.8±3.25mg/dL (P<0.001) and in two month FRUITITOP fed animal (**G-VI)** groups, it was 48.95±0.63mg/dL (P<0.001) compared to normal group (**G-III) (fig-11).**

In stage-I studies there is significant changes in average **triglyceride** level, In the one month FRUITITOP alone treated were 36.28±5.70 mg/dL (P<0.001), compared to normal (**G-II**) group was found to be 56.86±5.02 mg/dL. However stage-II studies TG level was found slightly raised in cyclophosphamide treated group 78.10±6.33 mg/dL (P<0.05), lower level in the triglycerides was observed in mices fed with one month FRUITITOP treatment group (**G-V)** 47.55±2.22 mg/dL (P<0.001), and two month FRUITITOP treatment group (**G-VI**) 38.27±1.59mg/dL (P<0.001) compared to normal group **G-III** (N2) **(fig-11).**

Preliminary stage-1 study there is no significant changes in **HDLc** level between normal (G-II) 49.92±2.87mg/dL and one month FRUITITOP alone treated group (G-1) 49.87±1.52mg/dL. During stage-II studies High density lipoprotein (HDLc) level was 58.59±4.95mg/dL in the normal animals and same in cyclophosphamide alone fed group was44.68±3.25mg/dL in this, there is a significant decrease (P< 0.05) was noticed compared to normal. No significant changes observed in HDLc level one month fruit treated (**G-V)** (49.8±2.40mg/dL) and two month fruits treated groups (**G-VI**) (48.5±1.41mg/dL) **(fig-11).**

Initial stage-1 study there is significant decrease in level of **LDLc** in one month FRUITITOP alone treated group (G-1) 25.41±3.65 mg/dL (P<0.001) compared to normal (G-II) 35.83±2.34mg/dL. During stage-II studies Low density lipoprotein (LDLc) level in normal (**G-III)** 37.94±2.40 mg/dL were compared to one month treated a group(**G-V**) with FRUITITOP followed by normal diet and two month FRUITITOP treated group**(G-VI)** were 28.25±2.62 mg/dL (P<0.001), 21.8±2.12mg/dL (P<0.001) respectively. There is no significance changes in cyclophosphamide treated group (**G-IV**) 42.64±3.35 compared to normal **(fig-11).**

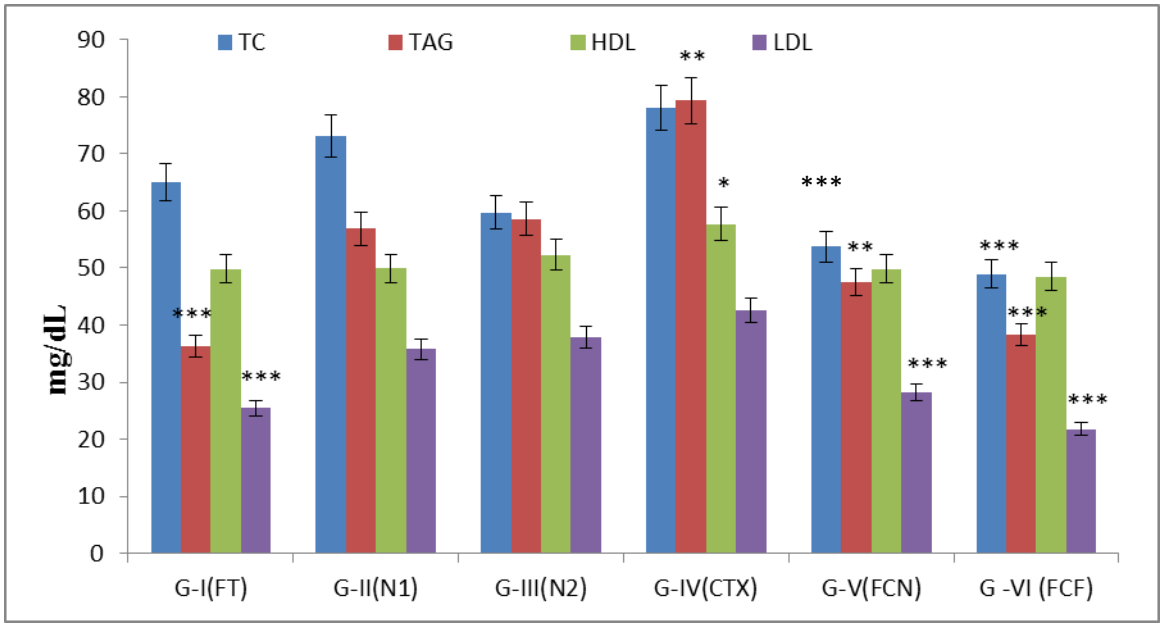


Fig-11, Effect of FRUITITOP on serum lipid profile

***** indicates P< 0.05, ****** indicates P< 0.01 and ******* indicates P< 0.001

11. IN-VIVO ANTIOXIDANT STUDIES

11a. HEPATIC ANTI-OXIDANT STATUS

Here in this study (Stage-1) there is significant increase in the anti-oxidant parameters like Catalase (P<0.001), Glutathione peroxidase (P<0.001) & Glutathione (GSH) (P< 0.001) and there is no significant changes observed in lipid peroxidation and super oxide dismutase compared to normal group (G-II). Similarly in stage-II study, GSH levels were found to be 1.841nmol/mg protein in the normal (G-III) However, there was a significant decrease in GSH levels observed with cyclophosphamide fed groups and increases in other treated groups. In contrast with the results of GSH levels, there was a significant increase in GPx values in G-IV (P< 0.05) & G-VI (P<0.001) no significant changes in G-V fed with one month FRUITITOP followed by second moth normal diet. Lipid peroxidation activity, where the increase LPO activity found in the G-IV CTX (P< 0.001) fed mice, and markedly reduced (P< 0.001) in other treated groups (G-V & G-VI). The level of SOD was raised in the Group-VI (P< 0.001) and

decreased in Group- IV (P< 0.05), However Group VI only shown the significant increases in Catalase activity (P< 0.001) compared to normal G-III (Table-18).

11b. **RENAL ANTI-OXIDANT STATUS**

In the (Stage-1) experiment there is significant increase in the anti-oxidant parameters like Catalase (P<0.001), SOD (P<0.01), LPO (P<0.001), & Glutathione (GSH) (P< 0.001) and there is no significant changes observed in GPx compared to normal group (G-II). But in two stage-II study, there is a significant reduction in the level of **Catalase** (P<0.001)**, GPx** (P<0.01)**, GSH** (P<0.001) **&** significant increase in **LPO** (P<0.001) levels were found in CTX treated groups compared to normal(Table-18). In two month FRUITITOP treated group (G-VI) shown significant increase in values of Catalase (P<0.001) GPx(P<0.001), GSH(P<0.001), SOD(P<0.01) & significant decrease in LPO(P<0.001) level compared to normal G-III. However group V shown only significant changes in Catalase (P<0.01), GPx(P<0.01) and LPO (P<0.001) levels(Table-19).

Table-18, LIVER (HEPATIC ANTI-OXIDANT STATUS)

LIVER	G-I FT	G-II N1	G-III N3	G-IV CTX	G-V FCN	G-VI FCF
CAT(U/mg protein)	8.902± 0.664***	6.98±0.653	6.535±0.761	5.603± 0.469^{ns}	7.234± 0.557^{ns}	9.999± 0.187***
GPX (U/mgprotein)	11.272± 0.800***	8.475±0.941	4.772±0.202	3.891± 0.410*	5.433± 0.650^{ns}	6.696± 0.978***
GSH(nmol/mg protein)	3.037± 0.021***	2.614±0.041	1.841±0.215	1.331± 1.024***	1.999± 0.092***	2.28± 0.087***
SOD(U/mg Protein)	0.322± 0.078^{ns}	0.251±0.021	0.22±0.051	0.134± 0.057*	0.296± 0.032^{ns}	0.361± 0.025***
LPO (nmol/mg. Protein)	0.482± 0.005^{ns}	0.584±0.008	0.863±0.094	0.931± 0.090***	0.498± 0.098***	0.362± 0.107***

* indicates P< 0.05, ** indicates P< 0.01 and ***indicates P< 0.001 ns- indicates non-significant (comparison is done between G-I1 Vs G-1 & G-III Vs (G-IV, G-V, G-VI)

Table-19, KIDNEY (RENAL ANTI-OXIDANT STATUS)

KIDNEY	G-I FT	G-II N1	G-III N3	G-IV CTX	G-V FCN	G-VI FCF
CAT (U/mg of protein)	3.847± 0.142***	2.65±0.269	3.206±0.550	2.933± 0.064***	3.887± 0.360**	4.124± 0.020***
GPX (U/mg protein)	6.012± 0.235	5.862±0.045	4.68±0.738	3.652± 0.750**	6.833± 0.260**	7.68± 0.488***
GSH (nmol/mg protein)	2.614± 0.045***	2.081±0.113	1.741±0.098	1.415± 0.135***	1.814± 0.054^{ns}	2.311± 0.069***
SOD (U/mg Protein)	0.295± 0.023**	0.229±0.010	0.261±0.049	0.233± 0.027^{ns}	0.286± 0.013^{ns}	0.317± 0.010**
LPO (nmol/mg. Protein)	0.382± 0.041***	0.485±0.054	0.716±0.065	0.879± 0.045***	0.614± 0.017***	0.538± 0.035***

* indicates P< 0.05, ** indicates P< 0.01 and *** indicates P< 0.001 ns indicates non-significant (comparison is done between G-I1 Vs G-1 & G-III Vs (G-IV, G-V, G-VI)

12. EFFECT OF FRUITITOP ON BONE MARROW CELLULARITY (BMC):-

There is a significant decrease in bone marrow cellularity (BMC) was found in group treated with cyclophosphamide (P< 0.001), compared to normal Group G-III (Table 0 & Fig0). The Groups I, V, VI treatment groups with FRUITITOP show no significant changes in bone marrow cellularity (Table-19).

Table: 20 - Effect of FRUITITOP on Bone Marrow Cellularity

	G-I FT	G-II N1	G-III N3	G-IV CTX	G-V FCN	G-VI FCF
	31st Day		61st Day			
BMC **('n' x 104 cells/femur)**	1389.87±66^{ns}	1316.42±38	1354.86±33	1143.98±56***	1301±31^{ns}	1332±42^{ns}

* indicates P< 0.05, ** indicates P< 0.01 and *** indicates P< 0.001 ns indicates non-significant (comparison is done between G-I1 Vs G-1 & G-III Vs (G-IV, G-V, G-VI)

13. HISTOPATHOLOGY

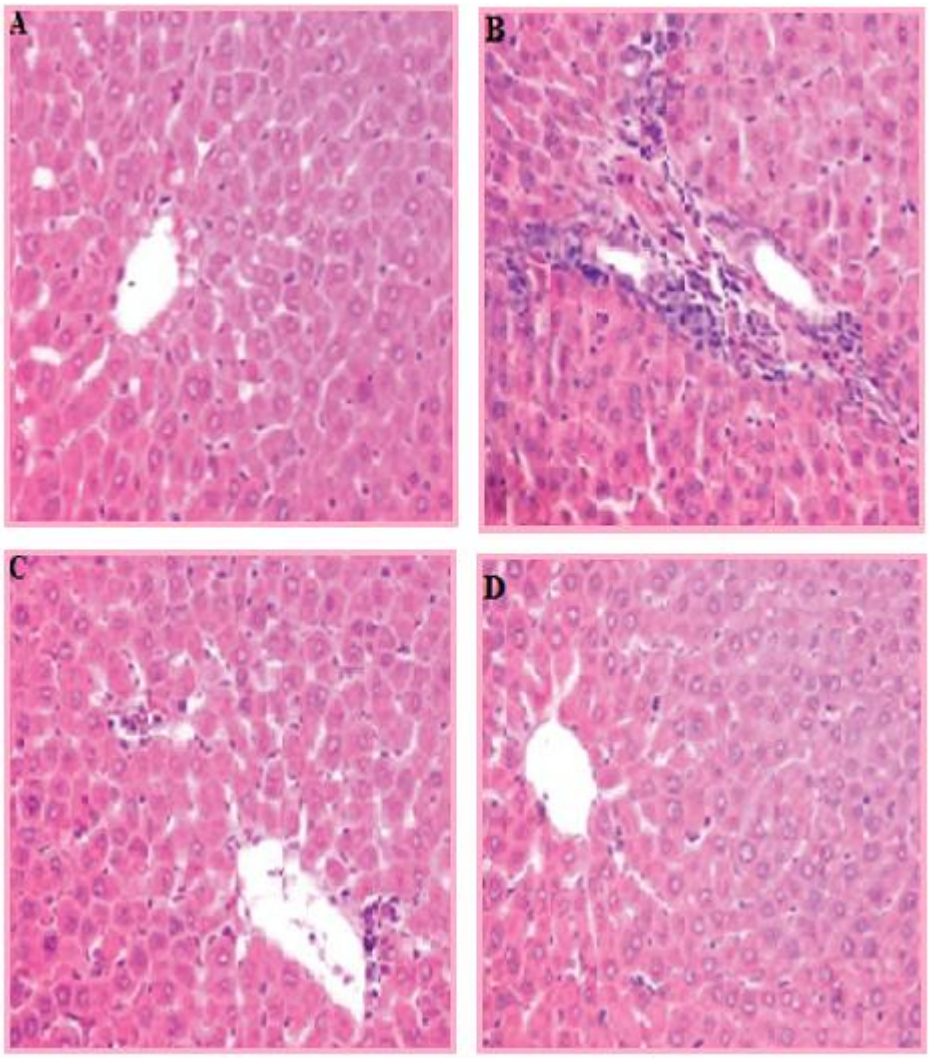


Fig.12. Histopathology of liver tissue Fig 5A. Normal liver histology of mice, 5B Cyclophosphamide fed animals 5C, One month FRUITITOP treated group followed by normal feed, 5D.Two months FRUITITOP treated group. Tissues were stained with Hand E stain and observerd under 20 X objective.

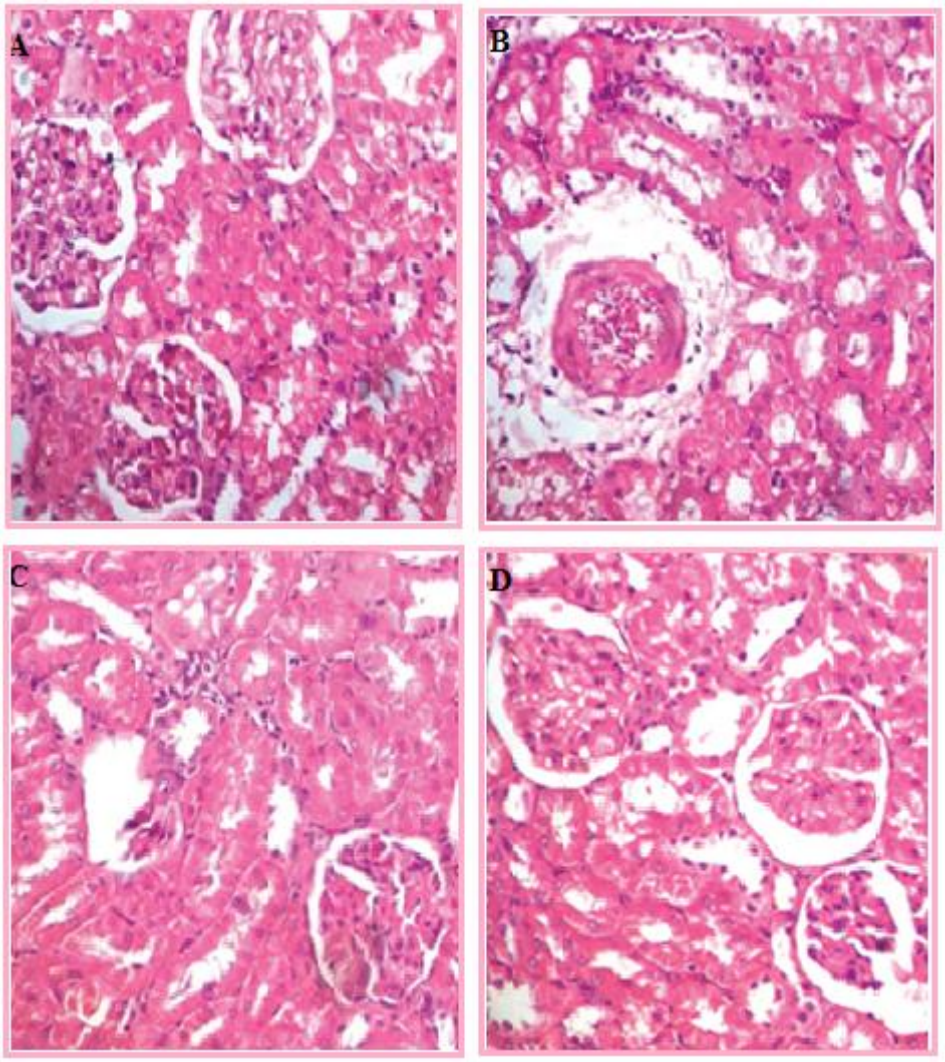


Fig.13. Histopathology of liver tissue Fig 5A. Normal liver histology of mice, 5B Cyclophosphamide fed animals 5C, One month FRUITITOP treated group followed by normal feed, 5D.Two months FRUITITOP treated group. Tissues were stained with Hand E stain and observerd under 20 X objective.

7. DISCUSSION

The proper maintain of lifestyle-food habit play important role in control socially, mentally and physically well-being state of life. Life style diseases are one of the utmost common upsetting disease affecting millions of individuals per year around the world. In life style diseases, cancer is the most threatening and second leading cases to cause death in human-beings.

The most threatening disease, cancer results from a different series of molecular altered mechanisms that essentially modify the common normal behaviour of cells in living system. The normal-healthy cells control cancer by preventing cell over-growth and the invasion of cancer cells to other tissue is restricted. Each and every disease will turn to cancer if left un-treatment for long period of time most probably, so early detection to be important for starting cancer treatment. But the treatment should not initiate till the occurrence of cancer cells is confirmed by a tissue (i.e., histologic) diagnosis. Treatment of cancer is the sequence of interventions such as psycho-social care, chemotherapy, surgical procedure and radiation therapy that is expected at curing the disease or lengthening life-time considerably while improving the patient's quality of life. The time period of treatment schedule depends on the stage of cancer, site of cancer and its state of progression.

Most of the chemotherapeutic medications are cytotoxic compounds that targeted towards rapidly growing oncogenic cells. Chemotherapy is the one of the most common option for the treatment of most of the cancers. More than 100 chemotherapy agents with altered way of mechanism are widely used, either as single form or combination therapy with other therapeutic drugs or treatments, treating specific forms of cancer.

In this present work anti-cancer drug cyclophosphamide (CTX) has been used to inducing stress. In-vivo Protective activity and anti-oxidant status of natural fruits pulp formulation "FRUITITOP" was studied against administration of cyclophosphamide (CTX) in Swiss-albino mice. Cyclophosphamide, an anti-cancer alkylating agent is probably the most frequently used standard drug in cancer research field. The main metabolites of cyclophosphamide (CTX) are Acrolein and Phosphoramide. Where phosphoramide can interact with DNA and a protein by alkylation of C-7 of guanine

nitrogen base, leads to formation of adducts (**Balu *et al*., 2001**). Phosphoramide mustard can cause myelo-suppression and haemorrhagic cystitis can be caused by the acrolein. The oxidative stress like loss of glutathione (GSH) and alkylation reactions at various nucleophillic sites in cell-particularly in the nucleus occur as results of high dose acrolein acute toxicity whereas, Low doses of acrolien inhibit proliferation of cells without affecting cell viability (**Horton *et al*., 1997**).

The "FRUITITOP" treated groups were found to shown that normal range of bone marrow cellularity (BMC) and total WBC count compared to cyclophosphamide treated group, this showing the protective effect and importance of fruits consumption as a protective agent. There is no significant change in the values of haemoglobin level in all the 6 groups.

It is estimated that one-third of all cancer and lifestyle disease could be prevented by improved diet, mostly improved consumption of fresh fruits, whole grains and fresh vegetables. So instead of consuming single fruits the consumption of combo of fruits will give essential nutrients, dietary anti-oxidant and different therapeutically significant nutra-ceuticals in healing and prevention of various diseases.

There is pharmacologic evidence that the biotransformation of CTX in to principal active moiety is mainly take place in liver (**Brock and Hohorst., 1967**). Biotransformation leads to formation of toxic metabolite, which may cause severe complications in hepatic system & renal system. Complication such as renal an hepatic system damage is mainly associated with the cell necrosis, the massive rise in tissue lipid peroxidation level and the depletion of tissue glutathione (GSH) (Sandy *et al*., 1998) and other important endogenous antioxidant level.

These results are in agreement with those of the current research work, wherein there was a significant depletion of GPx, GSH & SOD and a significant increase in lipid peroxides (MDA) in liver tissue homogenates of cyclophosphamide treated control group. But, in kidney tissue homogenates of cyclophosphamide treated control group there is a significant decrease in Catalase, GPx and GSH and significant increase in lipid peroxidation was found. While the lipid peroxidation level has been significantly reduced in both liver and kidney tissues of the one month and two month FRUITITOP treated group.

In order to determine whether the anti-oxidant properties of FRUITITOP are facilitated by the rise in in-vivo anti-oxidant enzyme activity, various antioxidant activity such as Catalase, SOD and GPx are measured in kidney and liver tissues of mice treated with one month and two month FRUITITOP. In normal cases without stress, the consumption of FRUITITOP formulation for one month significantly increases the level of Catalase, GPx and GSH in liver. But, in case of kidney there were a significant increase in Catalase, GSH, and SOD, compared to kidney there is no significant changes in the lipid peroxidation level in liver in normally.

In the current study one month FRUITITOP treatment mice group followed by normal diet shown significant increase in GSH level in liver tissue and significant increase in Catalase and GPx level were found in kidney tissue homogenate, and both kidney and liver homogenate shown significant decrease in lipid peroxidation value.

The study on FRUITITOP fed animal for two month shown significantly increased the Catalase, SOD and GPx activities of liver and kidney tissues.

Liver and Kidney damage was assessed by biochemical studies such as estimation of serum levels of AST & Creatinine respectively. Likewise, Merlin et al., 2011 reported the elevation of serum levels of many biochemical markers like aspartate transaminase (AST), triglycerides (TAG), cholesterol (TC) and bilirubin. Treatment of mice with cyclophosphamide increased the serum transaminase level in liver and kidney whereas animals pre-treated with FRUITITOP decreased the elevated levels of transaminases (AST) & Creatinine in a significant manner in one month and two month treated group.

Changes in the water consumption, feed intake, body weight and organ weight of animals were also analysed during the period to find out the effects of cyclophosphamide and FRUITITOP. There is a reduction in reduction in body weight is observed only in control groups and treated group, this may be due to variation in lipid profile level. The total cholesterol triglycerides and LDL_C level was significantly decreased in one month and two month FRUITITOP treated group. While in CTX treated group there is significant increase in TAG and LDL_C was noticed. No significant reduction in organ weight was noted.

In the histopathological studies of Liver and Kidney, FRUITITOP treated groups showed no significant structural damage when compared to the cyclophosphamide alone treated control group. In this observation it is assumed that FRUITITOP is highly significant to protect the KIDNEY AND LIVER form cyclophosphamide toxicity.

The antioxidants are recommended for general purpose to prevent chronic diseases. Uses of individual antioxidants were not proved properly but combinations of antioxidant have good pharmacological importance. In this present study fresh fruits combination has adequate nutrients, minerals and various phytonutrients are administered to mice has improved the antioxidants status. Antioxidants have the different mechanism to detoxify the toxic metabolites of drugs. It is thus possible that consumption of fruits in regular diet could be a dietary choice that can prevent various human ailments. So, these findings clearly demonstrated the chemo-protective activities of FRUITITOP in experimental mice models.

8. CONCLUSION

The data obtained from current study clearly indicates that regular supply of required amount of natural fruits combination will improve the antioxidant status that can challenge the secondary challenges shown by cyclophosphamide.

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10. APPENDIX

1. 0.06 M Phosphate buffer for SOD

NaH2PO4.2H2O-0.936g/100 ml distilled water

Na2HPO4.2H20-0.95g/100 ml distilled water

Take 8.5ml of NaH2PO4.2H2O, 91.5ml of Na2HPO4.2H20 and adjust the pH to 7.8.

2. 0.2 M phosphate buffer for glutathione estimation

NaH2PO4.2H2O-2.25g/100 ml distilled water

Na2HPO4.2H20-2.839g/100 ml distilled water

Take 5.3ml of NaH2PO4.2H2O, 94.7ml of Na2HPO4.2H20 and adjust the pH to 8.0.

3. 0.05M phosphate buffer for Catalase

KH2PO4 -0.618g/100 ml distilled water

Na2HPO4.2H20-1.78g /200 ml distilled water

Take 100ml of KH2PO4. , 155 ml of Na2HPO4.2H20 and adjust the pH to 7.0.